

---

Retrospective Theses and Dissertations

---

1979

## The Biodegradation of Vehicular Waste Petroleum in the Roadside Environment

Jesse W. Johnson  
*University of Central Florida*

 Part of the [Biology Commons](#)

Find similar works at: <https://stars.library.ucf.edu/rtd>

University of Central Florida Libraries <http://library.ucf.edu>

This Masters Thesis (Open Access) is brought to you for free and open access by STARS. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of STARS. For more information, please contact [STARS@ucf.edu](mailto:STARS@ucf.edu).

---

### STARS Citation

Johnson, Jesse W., "The Biodegradation of Vehicular Waste Petroleum in the Roadside Environment" (1979). *Retrospective Theses and Dissertations*. 427.  
<https://stars.library.ucf.edu/rtd/427>



THE BIODEGRADATION OF  
VEHICULAR WASTE PETROLEUM  
IN THE ROADSIDE ENVIRONMENT

BY

JESSE W. JOHNSON, JR.  
B.S., Florida Technological University, 1975

THESIS

Submitted in partial fulfillment of the requirements  
for the degree of Master of Science: Biology  
in the Graduate Studies Program of the College of Natural Sciences  
at the University of Central Florida; Orlando, Florida

Fall Quarter  
1979

## ABSTRACT

### Biodegradation of Vehicular Waste Petroleum in the Roadside Environment

Bacteria from dry and wet roadside environments were examined for the ability to degrade hydrocarbons. The kinds and numbers of bacteria observed were similar to those reported in other petroleum contaminated environments. Surface soils (top 2.5 cm) immediately adjacent to the highway pavement and the sediments of shallow drainage ditches contained the highest concentrations of petroleum degrading bacteria ( $9.8 \times 10^7$  CFU/g). Concentration and species diversity of petroleum degrading bacteria decreased with distance from the highway pavement. Chromatographic analysis of highway stormwater runoff and the soil in close proximity to the highway indicated the presence of complex hydrocarbon mixtures of vehicular origin. The concentrations of chloroform extractable hydrocarbons decreased with distance from the highway pavement. Hydrocarbon degradation rates in the roadside environment were determined by the oxidation of radiolabeled [ $1-^{14}\text{C}$ ] hexadecane. Roadside soil and water samples were incubated under nutrient enriched and in situ environmental conditions. Biodegradation rates in

environmental samples enriched with inorganic nutrients were 25-126 fold higher than the in situ rates. The highest in situ rates ( $92 \mu\text{g hexadecane g}^{-1} \text{ soil h}^{-1}$ ) occurred in wet surface soil (top 2.5 cm) immediately adjacent to the highway pavement. The findings of the investigation indicate that the roadside environment under study was a petroleum contaminated ecosystem in which biodegradation of hydrocarbon pollutants was greatly influenced by the design of the roadside drainage systems. Furthermore, petroleum degradation in roadside environments can be enhanced by construction of shallow drainage ditches which support aerobic microbial biodegradation.



## ACKNOWLEDGMENT

I wish to express my sincere appreciation to all those who assisted in the funding and national presentation of this research project. To the Florida Department of Transportation, University of Central Florida Graduate Studies and Research, In house award 182-000-080, Department of Civil Engineering and Environmental Sciences, Department of Biological Sciences, Student Government, State University System (STAR) Grant 111-620-003. To each of my committee members, friends, and especially to my family and wife, Debbie, for her support and understanding during my graduate study.

## TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGEMENT.....	iv
LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
LITERATURE REVIEW.....	1
I. <u>Contamination of the Roadside Environment With Vehicular Waste Petroleum</u> .....	1
II. <u>The Effects of Petroleum Pollution on the Roadside Environment</u> .....	6
A. <u>The effect of vehicular waste petroleum on aquatic roadside environments</u> .....	6
B. <u>The effect of vehicular waste petroleum on terrestrial roadside environments</u> .....	7
III. <u>The Mineralization of Hydrocarbons in the Roadside Environment</u> .....	9
A. <u>Autooxidation of vehicular hydrocarbons</u> .....	9
B. <u>Microbial biodegradation of vehicular hydrocarbons</u> .....	10
C. <u>Environmental factors influencing biodegradation</u> .....	14
IV. <u>Methods for Determination of Petroleum Bio-degradation Rates in the Roadside Environment</u> ...	16
A. <u>Biodegradation potential assessment</u> .....	16
B. <u>Biodegradation rate determination</u> .....	18
SECTION I: <u>THE PETROLEUM-DEGRADING POTENTIAL OF BACTERIA FROM ROADSIDE ENVIRONMENTS</u> .....	20
<u>Introduction</u> .....	21
<u>Materials and Methods</u> .....	23
<u>Sample sites</u> .....	23
<u>Sampling methods</u> .....	23

<u>Media</u> .....	24
<u>Gas-liquid chromatography</u> .....	25
<u>Soil analysis</u> .....	26
<u>Results</u> .....	27
<u>Effects of rainfall</u> .....	27
<u>Soil and stormwater extraction</u> .....	27
<u>Inorganic nutrient concentration</u> .....	39
<u>Evaluation of the biodegradation potential</u> .....	44
<u>Discussion</u> .....	46
SECTION II: <u>ESTIMATION OF THE OPTIMUM AND IN SITU HYDROCARBON DEGRADATION RATES IN THE ROADSIDE ENVIRONMENT</u> .....	50
<u>Introduction</u> .....	51
<u>Materials and Methods</u> .....	52
<u>Sample sites and methods</u> .....	52
<u>Hexadecane-kerosene assay</u> .....	52
<u>Optimum rates determination</u> .....	55
<u>In situ rates determination</u> .....	56
<u>Results</u> .....	58
<u>Comparison of hexadecane-kerosene biodegradability</u> .....	58
<u>Optimum [<math>1-^{14}\text{C}</math>] hexadecane rates</u> .....	58
<u>Comparison of optimum and in situ degradation</u> .....	65
<u>Discussion</u> .....	67
GENERAL DISCUSSION.....	72
GENERAL SUMMARY.....	75
LITERATURE CITED.....	78

## LIST OF TABLES

### TABLES

	<u>page</u>
1. <u>Average amounts of major classes of hydrocarbons and related compounds present in vehicular petroleums</u> .....	2
2. <u>Relative biodegradability of various hydrocarbons substrates</u> .....	11
3. <u>Microorganisms most frequently isolated from petroleum contaminated environments</u> .....	13
4. <u>Concentration of chloroform extractable hydrocarbons in roadside soil</u> .....	36
5. <u>Predominant hydrocarbon bacteria isolated from the roadside environment</u> .....	45
6. <u>Comparison of kerosene and hexadecane biodegradation under optimum conditions</u> .....	59
7. <u>Comparison of hydrocarbon biodegradation under optimum and in situ conditions</u> .....	64



## LIST OF FIGURES

### FIGURES

	<u>page</u>
1. <u>Typical roadway, shoulder, and ditch environments</u> .....	5
2. <u>The influence of rainfall and distance from the highway pavement on the concentration of hydrocarbon utilizing bacteria</u> .....	29
3. <u>Gas-liquid chromatographic tracing of the chloroform extractable hydrocarbons present in one gram of roadside soil</u> .....	31
4. <u>Gas-liquid chromatographic tracing of the chloroform extractable hydrocarbons present in one liter of highway stormwater runoff</u> .....	33
5. <u>Gas-liquid chromatographic tracings of weathered and biodegraded kerosene</u> .....	35
6. <u>The effect of distance from the pavement, bacterial concentration, total nitrogen concentration, and phosphate concentration on the biodegradation of kerosene by soil collected from the dry site during the dry season</u> .....	38
7. <u>The effect of distance from the pavement, bacterial concentration, total nitrogen concentration, and phosphate concentration on the biodegradation of kerosene by soil collected from the dry site during the rainy season</u> .....	41
8. <u>The effect of distance from the pavement, bacterial concentration, and phosphate concentration on the biodegradation of kerosene by surface water and sediment collected from the wet site ditch</u> .....	43
9. <u>An apparatus for determining the optimum relative biodegradability of a hexadecane-kerosene mixture</u> .....	54



10. <u>Gas-liquid chromatographic tracing</u> <u>of a sterile weathered hexadecane-kerosene</u> <u>mixture following 70 h. incubation.....</u>	61
11. <u>Gas-liquid chromatographic tracing of</u> <u>biodegraded hexadecane-kerosene mixture</u> <u>following 70 h. incubation.....</u>	63

## LITERATURE REVIEW

### I. Contamination of the Roadside Environment With Vehicular Waste Petroleum

The roadside environment is defined as the terrestrial and aquatic ecosystems of the land area extending from the highway pavement to the right-of-way boundary line. These areas are constructed to receive highway stormwater runoff and play a decisive role in the removal of highway related pollutants. Highway related pollutants stem from automotive emissions which include heavy metals, noxious gases, and waste petroleum lubricants (15,33).

The investigation presented in the following sections involves the removal of vehicular waste petroleum by microbiological degradative processes. The design of roadside drainage systems influences these biodegradative processes and may enhance or retard the mineralization of highway related petroleum pollutants.

Vehicular waste petroleum is comprised of hydraulic fluids, automotive lubricants, crankcase oil, and hydrocarbon components of automotive exhaust (24). Highway related petroleum pollutants are complex mixtures of aliphatic and aromatic hydrocarbons refined from crude oil (Table 1).

TABLE 1. Average amounts of major classes of hydrocarbons  
and related compounds present in vehicular petroleum

Component	Percentage
Aliphatic or paraffinic (alkanes)	15-35
Cycloparaffinic (cycloalkanes; napthenes)	30-50
Aromatic (benzene and polynuclear series)	5-20
Asphaltic (asphaltenes; heterocyclic compounds with oxygen, sulfur, or nitrogen)	2-15

From Zobell (35).

Automotive lubricants are deposited on highway surfaces by grease fittings, machine joints and leaking gasket seals. The volatile hydrocarbon fractions evaporate and contribute to photochemical smog reactions in the upper atmosphere (16). Non-volatile hydrocarbons remain on the highway surface as grease and oil deposits.

Highway surfaces are designed to drain stormwater rapidly. Street surface contaminants including waste petroleum deposits become constituents of highway stormwater and are removed from the pavement. Vehicular waste oil has been identified as the major organic constituent of highway stormwater runoff (24). The stormwater is discharged into the adjacent roadside area. The design of the roadside drainage system determines the direction of stormwater and petroleum pollutant flow. Stormwater can be channeled into adjacent waterways and holding ponds or forced to flow overland and infiltrate into surface soil (Fig. 1).

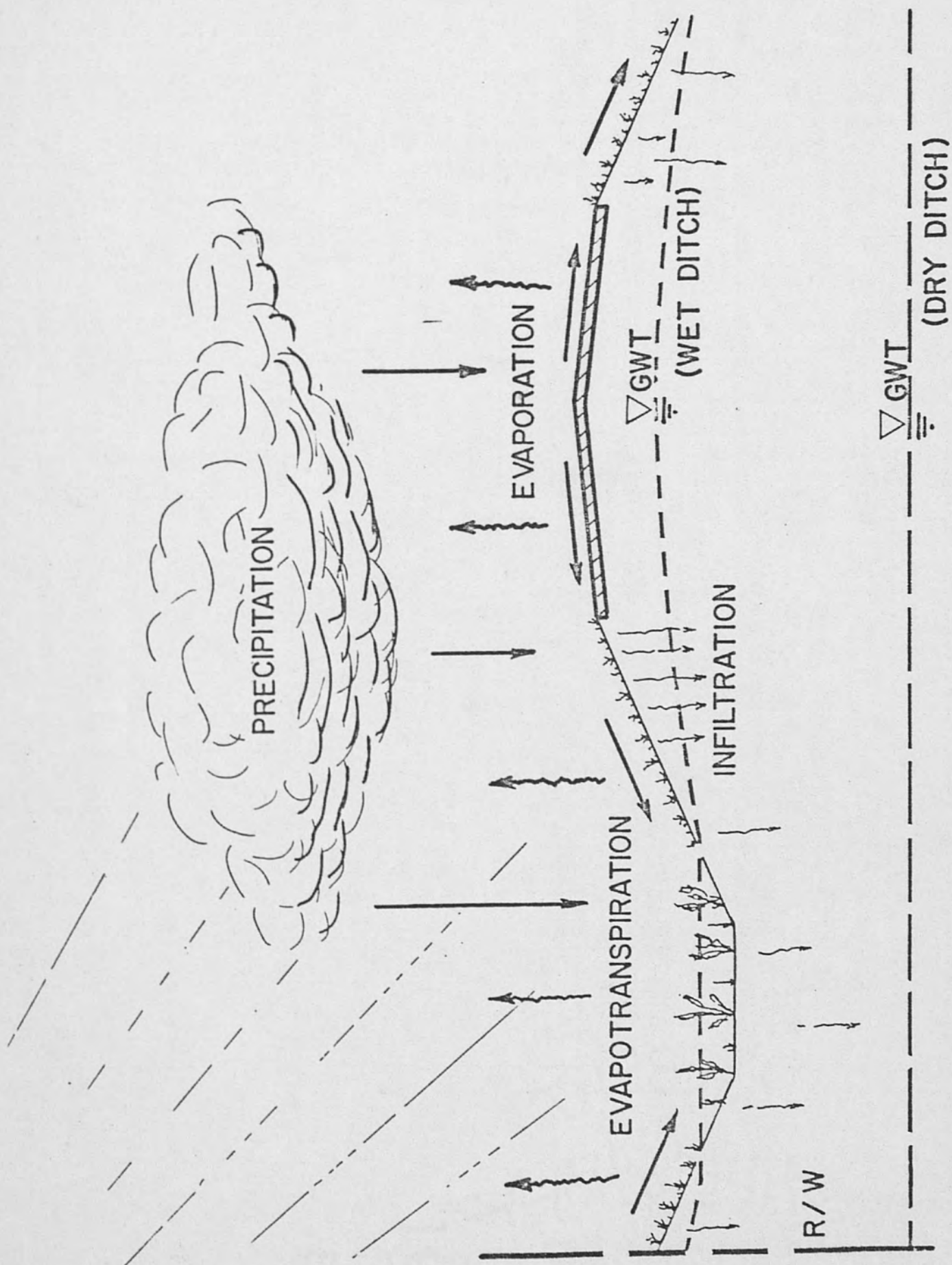
The ultimate fate of vehicular waste petroleum is mineralization by chemical and microbiological processes. The design and construction of roadside drainage systems influences environmental parameters (e.g., oxygen, moisture, and available nutrients) which influence degradative processes. Thus, roadside design determines the fate of highway petroleum pollutants.



FIG. 1. Typical roadway, shoulder, and ditch environments.

Reproduced from Wanielista et al. 1978. Shallow-water roadside ditches for stormwater purification, p. 3. State University System: Star Program, Florida Technological University, Orlando, Florida (33).





## II. The Effects of Petroleum Pollution on the Roadside Environment

The introduction of vehicular waste oil into the roadside environment produces several physico-chemical and biological alterations.

### A. The effect of vehicular waste petroleum on aquatic roadside environments

Vehicular waste petroleum enters roadside aquatic ecosystems as a constituent of stormwater runoff. Petroleum components are less dense than water and form a thin layer of oil across the surface of the drainage ditch. In ditches or holding ponds which are designed to retain water, the oily layers persist. Waste petroleum combines with suspended organic materials which form an impermeable layer over the drainage ditch surface. Oxygen exchange and sunlight penetration is reduced. These disturbances result in several ecological changes (3).

Decreased oxygen exchange lowers dissolved oxygen concentration and increases the biological oxygen demand. Decreased sunlight penetration reduces photosynthetic activity which further decreases the dissolved oxygen

concentration. When the surface oil layer is not disrupted by wind or water currents, the available oxygen beneath the oil layer is rapidly depleted by aerobic bacteria and higher eucaryotic organisms. Anaerobic conditions become established accompanied by low oxidation reduction potentials and decreases in pH. Thus, aerobic biodegradative processes are greatly impaired or completely cease. The overall effect is eutrophy. Organic sediments accumulate unoxidized materials, e.g., vehicular petroleum pollutants. The roadside aquatic ecosystem loses its aesthetic appearance and becomes "stagnant."

Anaerobic conditions in roadside aquatic environments can be circumvented by designing shallow roadside drainage ditches in which stormwater percolates into surface soil. Proper stormwater drainage reduces the formation of surface oil slicks and accumulation of hydrocarbons in aquatic ditch sediments (33).

B. The effect of vehicular waste petroleum  
on terrestrial roadside environments

In roadside areas in which stormwater runoff is forced to drain overland, waste petroleum infiltrates into surface soil (top 2.5 cm) and combines with soil particles. The presence of vehicular waste petroleum causes microbiological changes in the indigenous soil microflora. Microorganisms capable of utilizing hydrocarbons as substrates for

growth have a selective advantage over other microbial populations. Petroleum utilizing microbial populations increase in concentration with increasing amounts of hydrocarbons (33).

Roadside surface soils exposed to stormwater runoff support active aerobic biodegradation. Vehicular waste petroleum is actively degraded by the soil microflora thus preventing accumulation. Extreme environmental changes which accompany the accumulation of petroleum pollutants, e.g., anaerobic conditions, eutrophication, are not associated with surface soil adjacent to highways.



### III. The Mineralization of Hydrocarbons in the Roadside Environment

The removal of vehicular waste petroleum from the roadside environment is accomplished by the interaction of chemical autooxidative and microbial biodegradative processes.

#### A. Autooxidation of vehicular hydrocarbons

Autooxidation is a complex chemical oxidation reaction which is initiated thermally or photochemically by the formation of free-radical chemical intermediates (17). The formation of the free-radicals produce a chemical chain reaction which in turn produces additional reactive intermediates. These react with molecular oxygen ( $O_2$ ) resulting in the partial or complete mineralization of the hydrocarbon molecule.

Sunlight, temperature, partial pressure of oxygen and oil density influence the degree of hydrocarbon autooxidation. Autooxidation reactions occur most readily in thin layer oil slicks and oil emulsifications where the partial pressure of oxygen is increased (35). Autooxidation



reactions have been observed in virtually all hydrocarbon components of refined petroleum (17).

Partial oxidation of vehicular fuels and lubricants by autooxidation reactions form aldehydes, ketones, esters and organic acids (17). These partially oxidized compounds are less recalcitrant than their hydrocarbon precursors. Recalcitrant hydrocarbons, e.g., aromatics and cycloalkanes (Table 2), represent 30-50% of the hydrocarbons present in vehicular lubricants (Table 1). The combined interaction of autooxidation and microbial degradation is essential for optimum mineralization of the recalcitrant hydrocarbons which enter the roadside environment.

#### B. Microbial biodegradation of vehicular hydrocarbons

Vehicular waste petroleum fractions enter the roadside soil and aquatic systems where they become substrates for microorganisms. The hydrocarbon components of petroleum supply both energy and carbon skeletons for microbial metabolism. The microbial cell oxidizes the hydrocarbons by oxygenase enzymes which catalyse the reaction of the hydrocarbon molecule with molecular oxygen. This biochemical reaction provides the cell with reducing power and chemical energy for other metabolic processes. The biochemical oxidation of hydrocarbons is the biological basis for petroleum mineralization. Reviews describing this biochemical

TABLE 2. Relative biodegradability of  
various hydrocarbon substrates

Recalcitrance <sup>a</sup>	Hydrocarbon	Specificity <sup>b</sup>
	Normal alkanes C <sub>10</sub> - C <sub>19</sub>	
	Straight chain alkenes C <sub>12</sub> - C <sub>19</sub>	
	Gases C <sub>2</sub> - C <sub>4</sub>	
	Alkanes C <sub>5</sub> - C <sub>9</sub>	
	Branched alkanes C <sub>4</sub> - C <sub>12</sub>	
	Alkenes C <sub>3</sub> - C <sub>11</sub>	
	Branched alkenes	
	Aromatics	
↓	Cycloalkanes	↑

<sup>a</sup> The number of microorganisms isolated that would grow on the substrates listed from top to bottom.

<sup>b</sup> Any organisms isolated on the compounds further down the list would generally grow on those above.

From Perry, J. J. and C. E. Cerniglia (18).

degradative process have been published (2,8).

Microorganisms which degrade hydrocarbons are widely distributed in soil and water. However, the ability to biochemically oxidize hydrocarbons is restricted to a relatively small group of microorganisms in nature. The hydrocarbon utilizing microorganisms most frequently isolated from oil contaminated environments are strict aerobic bacteria (Table 3). These aerobic microorganisms are very versatile at biodegradation. Some members of the genus Pseudomonas are capable of growth on over 80 individual carbon sources in minimal media (4).

The biodegradation process involves chemical oxidation of a highly reduced molecule. The thermodynamics of such reactions require the presence of molecular oxygen and/or a positive oxidation-reduction potential which favors the formation of oxidized products. Microbial oxidation of hydrocarbons in anaerobic sediments does not appear feasible at any appreciable rate. However, a highly specialized group of anaerobic bacteria, e.g., Desulfovibrio sp., are capable of reducing sulfate and assimilating hydrocarbons. This degradative process requires sulfate which is reduced to hydrogen sulfide as the hydrocarbon becomes oxidized (3). Hydrocarbon mineralization studies which utilized sensitive short term  $^{14}\text{C}$  radioassays did not detect active anaerobic hydrocarbon oxidation in organic sediments (2). Thus, it has been concluded that mineralization of hydrocarbons in an

TABLE 3. Microorganisms most frequently isolated  
from petroleum contaminated environments

Microorganism	Hydrocarbons Utilized	References
<u>Pseudomonas sp.</u>	Paraffins, cycloalkanes, naphthenes, phenols, m-creosols, and asphalt	4
<u>Alcaligenes sp.</u>	Fatty acids, alcohols, partially oxidized alkanes	9
<u>Micrococcus sp.</u>	Paraffins, phenols	9
<u>Nocardia sp.</u>	Paraffins, aromatics	20
<u>Flavobacterium sp.</u>	Paraffins, aromatics	2
<u>Corynebacterium sp.</u>	Paraffins	29
<u>Arthrobacter sp.</u>	Benzene extractable hydrocarbons	2
<u>Acinetobacter sp.</u>	Paraffins, aromatics	27
<u>Vibrio sp.</u>	Paraffins, aromatics	6
fungi	Paraffins, aromatics	10



anaerobic environment is an extremely slow process (2).

The removal of hydrocarbons from the roadside environment is predominantly an aerobic microbial degradative process. Anaerobic roadside environments support the accumulation of vehicular waste oil and retard the rates of aerobic microbial biodegradation.

### C. Environmental factors influencing biodegradation

Physical and chemical factors which affect microbial growth influence the rate of oil biodegradation in the roadside environment. Dissolved oxygen, temperature, available water and inorganic nutrient concentration all play the major role in determining the rates of petroleum biodegradation.

Free or dissolved oxygen is essential for active aerobic microbial degradation (35). Microbial degradation rates in the roadside environments are highest in the surface of soil and water (top 2.5 cm) where oxygen is readily available. Degradation rates decline in water-saturated soils and sediments where oxygen cannot perfuse.

Temperature affects the kinetics of biochemical reactions and thus influences biodegradation rates. The optimum temperature for growth of most aerobic petroleum utilizing bacteria is 23 to 25° C (34). In cold climates (<4° C) temperature is the rate limiting factor in petroleum degradation



(34). Petroleum biodegradation rates in subtropical climates in which mean climate temperatures approach optimum exceed the rates of degradation in temperate climates. In roadside environments of temperate climates, temperature can become a limiting factor of vehicular petroleum degradation (34).

Water availability influences microbial growth and thus affects petroleum biodegradation. In arid roadside soils, bacterial metabolic activity decreases and microbial populations decline. Increasing the moisture content solubilizes nutrients and results in a surge of microbial growth and metabolic activity. Roadside environments with increased soil moisture have higher rates of oil degradation than arid soils. During rainy months, the bacterial populations and biodegradation rates increase.

In petroleum contaminated environments inorganic nutrients are rapidly assimilated and the petroleum utilizing microbial populations become nutrient limited. As a result, the rates of hydrocarbon degradation decline (2). In the roadside environment, the carbon to nitrogen imbalance and phosphorus limitation is ameliorated by the addition of inorganic nutrients present in highway stormwater runoff (24). In the presence of adequate inorganic nutrients, microbial populations remain active during intervals of heavy hydrocarbon loading.

IV. Methods for Determination of Petroleum  
Biodegradation Potentials and Degradation Rates  
in the Roadside Environment

Assessment of the biodegradation of hydrocarbons in a petroleum contaminated environment can be determined by a variety of laboratory methods. The methods employed in this study have been utilized in other petroleum contaminated environments by other investigators and proven effective (7,30,31).

A. Biodegradation potential assessment

Determination of the hydrocarbon biodegradative potential involves incubation of environmental samples under optimum environmental conditions, e.g., oxygen, temperature, moisture, and nutrient enrichment. Predetermined amounts of hydrocarbons identical to, or representative of the petroleum pollutants under study are added to each environmental sample. The environmental samples are incubated for a given time period and the residual amount of hydrocarbon remaining is quantitatively and/or qualitatively determined.

Two methods are frequently used to quantitatively and/or qualitatively determine residual hydrocarbons following

biodegradation: gravimetric (19) and gas-liquid chromatographic analysis (11).

Gravimetric analyses measure differences in hydrocarbon substrate weight between biodegraded samples and sterile controls. Petroleum residues of environmental samples are extracted with a volatile solvent, dried under nitrogen and weighed on an analytical balance. Gravimetric analyses are quantitative measurements and are useful in comparing the relative biodegradative effectiveness of environmental samples. However, this method does not offer qualitative information concerning the biodegradation of specific petroleum fractions.

Gas-liquid chromatographic analyses offer both qualitative and quantitative biodegradation assessment (6). Petroleum residues are extracted and injected into an instrument containing columns packed with an inert solid support material coated with a "liquid phase," e.g., diatomaceous earth/dimethyl-silicon. The columns are enclosed in a temperature controlled oven with an ambient temperature sufficient to volatilize all components of the residue. The vaporized hydrocarbons are passed through the column by an inert carrier gas, e.g., helium or nitrogen. The hydrocarbons interact and adsorb to the liquid phase dependent upon their respective chemical and physical properties. The residue is resolved into individual hydrocarbon components which elute at different time intervals (retention times). As each component leaves the column it

is electrically detected by a flame ionization detector and recorded. Residual hydrocarbon concentrations in the mg/L range are detected by this methods. Hydrocarbon component identification and concentration is obtained by standard concentration curves and retention time evaluation.

Gas-liquid chromatographic methods are superior in sensitivity to gravimetric methods and offer qualitative analysis. Thus, gas-liquid chromatographic methods were selected for the hydrocarbon analysis in this study.

#### B. Biodegradation rate determination

Biodegradation rate determinations are essential in evaluating petroleum mineralization. Rate determination methods involve the addition of hydrocarbon substrates to environmental samples and quantitatively measuring the degree of mineralization during an established time interval. Biodegradation rates may be determined under a variety of laboratory controlled environmental conditions. Maximum biodegradation rates are measured by incubating environmental samples under conditions of artificial nutrient enrichment and aeration. Optimum rate studies provide valuable information concerning biodegradation potential but do not assess actual environmental biodegradation rates. Actual environmental rates are assessed by the incubation of hydrocarbon substrate in environmental samples under in situ



environmental conditions.

The most widely accepted method for determining hydrocarbon biodegradation rates employs  $^{14}\text{C}$ -radiolabeled hydrocarbons (2). This method quantitatively measures the mineralization rate of  $^{14}\text{C}$ -hydrocarbon to  $^{14}\text{CO}_2$ .  $^{14}\text{C}$ -hydrocarbons are incubated under laboratory controlled conditions. The  $^{14}\text{CO}_2$  is directly trapped into a basic solution and counted by liquid scintillation procedures (32). Radiolabeled hydrocarbon assays are very sensitive methods,  $\mu\text{M}$  detection, which permit the study of oxidation rates under a variety of laboratory controlled conditions. These methods provide a direct comparison of both biodegradation rate potential and in situ rate determination. Radiolabeled  $[1-^{14}\text{C}]$  hexadecane was utilized to determine the biodegradation rate in this study.

SECTION I: THE PETROLEUM-DEGRADING POTENTIAL OF BACTERIA  
FROM ROADSIDE ENVIRONMENTS

Bacteria from dry and wet soils exposed to highway stormwater runoff were examined for their ability to degrade a representative mixture of hydrocarbons present in vehicular oil. Surface soils 0.76 m from the roadside contained twice as many aerobic petroleum degrading bacteria as soils 6.0 m from the highway pavement. Bacteria present in soil near the edge of the highway and in sediments of shallow drainage ditches produced significantly greater degradation of hydrocarbons when compared with bacteria of dry soils and soils not directly exposed to runoff. Under optimum environmental conditions, 89.6% of a 20.0 g/liter hydrocarbon mixture was degraded in 60 days by bacteria from roadside soils. The number and kinds of microorganisms associated with roadside soils are similar to those found in other petroleum contaminated environments.

## Introduction

The environmental impact of petroleum pollution upon aquatic ecosystems has resulted in extensive research into the microbial biodegradation of hydrocarbons. Several studies have estimated the hydrocarbon degradation potential of water and sediment of estuary, marine and freshwater environments (21,28,30,31). However, very few have studied the biodegradation of waste oil present in the stormwater runoff of streets and highways.

Hydrocarbons present in stormwater runoff result from the deposition of vehicular lubricants and fuels onto the street surface. Analysis of roadside runoff from residential and industrial areas indicate automotive grease and waste oil as the major organic constituents (24). The primary hydrocarbon contaminants include crankcase oil and the non-volatile components of engine fuel. Of the estimated  $1.51 \times 10^9$  liters of waste crankcase oils produced annually in the U.S., approximately  $5.0 \times 10^8$  liters are lost through leakage and combustion (15).

Vehicular waste oils originate from machine joints, worn seals, grease fittings, and from automotive exhaust as incomplete fuel combustion (24). Volatile components of waste oils and fuels evaporate into the upper atmosphere

and contribute to photochemical smog processes (16). Non-volatile hydrocarbons remain deposited on the pavement surface and become constituents of stormwater runoff. Stormwater runoff transports the waste oils into roadside soils and aquatic systems (14,26). The design of drainage systems, roadside ditches and runoff holding ponds can enhance or retard microbial degradation of the waste oils introduced into the roadside environments.

The objective of this study was to describe the petroleum degrading bacterial concentration in the roadside environment, and to determine the relative effectiveness of roadside soil and water to biodegrade hydrocarbons present in highway stormwater runoff.

The data indicated that the concentration of petroleum utilizing bacteria was highest in the vicinity of highway stormwater discharge. Soils of increased moisture content near the highway pavement and the shallow aquatic ditches proved most effective in degrading hydrocarbons. Arid soils and the anaerobic sediments of aquatic ditches were the least effective in the mineralization of hydrocarbons.



### Materials and Methods

Sample sites. Two roadside areas along Interstate 75 near Titusville, Florida were selected. A dry roadside sampling region 150 m in length extending 20 m from the pavement was chosen as a representative dry site. This site was directly exposed to waste petroleum present in highway stormwater runoff. Stormwater flowed over the surface soil and infiltrated into the ground water 2 m below. Samples were also collected from a roadside ditch 6 m from the pavement, 150 m in length, and 3 m in width. Highway stormwater was channeled directly from the road surface into the wet site ditch through concrete pipes and flumes. Water in the wet site ditch was 1 m in depth throughout the sampling period. The two sites were 5 km apart. Neither sampling region was exposed to agricultural, residential nor industrial influences. Traffic volume during the sampling period was determined to be  $1.24 \times 10^4$  axles per day (personal communication, Florida Department of Transportation, Tallahassee, Florida).

Sampling methods. Composite soils and water samples were collected in sterile one liter jars, transported on ice and processed the day of collection. Composite surface

soil samples (top 2.5 cm) were collected at various distances from the pavement with sterile trowels and jars. Composite water samples were collected from the wet site by submerging sterile jars. Sediment samples were collected by submerging a closed sterile jar and opening the lid of the jar prior to collecting the bottom sediments.

Media. The basal salts medium for the isolation and enumeration of petroleum utilizing aerobic microorganisms contained: 0.05 g  $\text{FeCl}_3$ , 0.05 g  $\text{KH}_2\text{PO}_4$ , 0.5 g  $\text{MgSO}_4$ , 0.6 g  $\text{NaCl}$ , 0.05 g  $\text{NaH}_2\text{PO}_4$ , 1.0 g  $\text{NH}_4\text{Cl}$ , 15.0 g agar, and 1000 ml distilled water. The pH was adjusted to 7.2 prior to autoclaving. The media was sterilized and dispensed into plastic petri plates (100 x 10 cm). Sterile kerosene (0.2 ml) was overlayed onto the surface of the agar prior to inoculation. Enumeration of bacteria was determined by the spread plate count technique in replicates of four. Bacterial numbers were expressed as colony-forming units per gram of soil or milliliter of water. Bacterial identification was accomplished using Bergey's Manual of Determinative Bacteriology (4). All plates were incubated at 23° C.

The biodegradation potential was determined by measuring the ability of microorganisms present in the samples to degrade a representative hydrocarbon mixture (kerosene) under optimum environmental conditions. One hundred milliliters of basal salts solution was placed into 250 ml Erlenmeyer flasks and buffered with 2.0 ml of 0.5 M  $\text{NaH}_2\text{PO}_4$ . The

pH was adjusted to 7.2 with 1.0 N NaOH. The flasks were fitted with cotton stoppers and autoclaved. Sterile kerosene (2.0 g) was added to each flask. One gram or 1.0 ml of composite sample was added to the flask and incubated at 23° C for 60 days on a rotary shaker at 100 rpm with a 5 cm throw. Volatilization and weathering effects were determined using sterile control flasks containing basal salts and kerosene.

After a 60 day incubation period, the contents of each flask were emptied into 250 ml separatory funnels and extracted with 20.0 ml Nanograde chloroform (Mallinckrodt). The chloroform layer containing the residual kerosene was filtered through a 10 cm, 0.45  $\mu$  Fluoropore filter (Millipore Co.) to remove the soil and bacterial cell mass. Twenty (20.0 ml) of chloroform was utilized to extract the contents of the glass separatory funnel and filtering flask. The chloroform-extracted residue was placed in a 500 ml round bottom flask and flash evaporated at 55° C to a constant volume of 5.0 ml. Five  $\mu$ l of the concentrated extract were injected into a gas-liquid chromatograph. The concentration of kerosene remaining after degradation was quantitatively determined by integration of the area under the chromatographic tracing.

Soil and water samples from the roadsides exposed to petroleum were directly extracted using the above procedure.

Gas-liquid chromatography. Chromatographic analysis of hydrocarbons was determined on a Hewlett Packard 7260A

gas-liquid chromatograph equipped with a dual flame ionization detector. Stainless steel columns, 3 m x 4 mm, containing 10% SP-2100 on 80/100 Supelcoport (Supelco, Inc.) were utilized to resolve the hydrocarbon components of kerosene and waste petroleum. Separation was achieved by the following temperature program: initial column temperature 50° C, 2 min. post injection time, temperature increased to 270° C at a rate of 10° C per min. final temperature held for 10 min. Other instrument parameters and settings included the following: a nitrogen carrier gas, 40 cc per min., injection temperature, 310° C, detector temperature 300° C, air flow rate, 425 cc per min., hydrogen flow rate, 40 cc per min., recorder chart speed, 1.25 cm per min.

Soil analysis. Total nitrogen and total phosphate analyses were performed on the roadside samples according to Standard Methods for the Examination of Water and Wastewater (1). Concentrations were expressed in mg per liter.



## Results

Effects of rainfall. The concentration of hydrocarbon utilizing bacteria present in the top 2.5 cm of roadside soil decreased with distance from the pavement ( $r = -0.86$ ,  $p < 0.05$ , Fig. 2). Bacterial concentrations increased significantly as rainfall increased (Fig. 2).

Soil and stormwater extraction. Chromatographic tracings of soil and highway stormwater extracts (Fig. 3 and 4) indicated the presence of hydrocarbons similar to sterile weathered kerosene (Fig. 5). The tracing of chloroform extracted stormwater represented a hydrocarbon concentration of 0.62 mg/liter (Fig. 4). The concentration of extractable hydrocarbons in surface soil decreased with distance from the pavement ( $r = -0.91$ ,  $p < 0.01$ , Table 4). Hydrocarbon concentrations present at the edge of the pavement were 76.7% higher than in soil collected 6.0 m from the pavement.

The concentration of petroleum utilizing bacteria, nitrogen, phosphate, and the ability to degrade kerosene under optimum conditions, decreased with distance from the pavement at the dry site (Fig. 6). The highest degradation during the dry season was associated with surface soil near the edge of the pavement.

FIG. 2. The influence of rainfall and distance from the highway pavement on the concentration of hydrocarbon utilizing bacteria. Symbols: ● , mean rainfall; ○ , bacterial concentration at 0.0 m; □ , bacterial concentration at 0.75 m; ■ , bacterial concentration at 1.5 m; ▲ , bacterial concentration at 3.0 m; △ , bacterial concentration at 6.0 m.

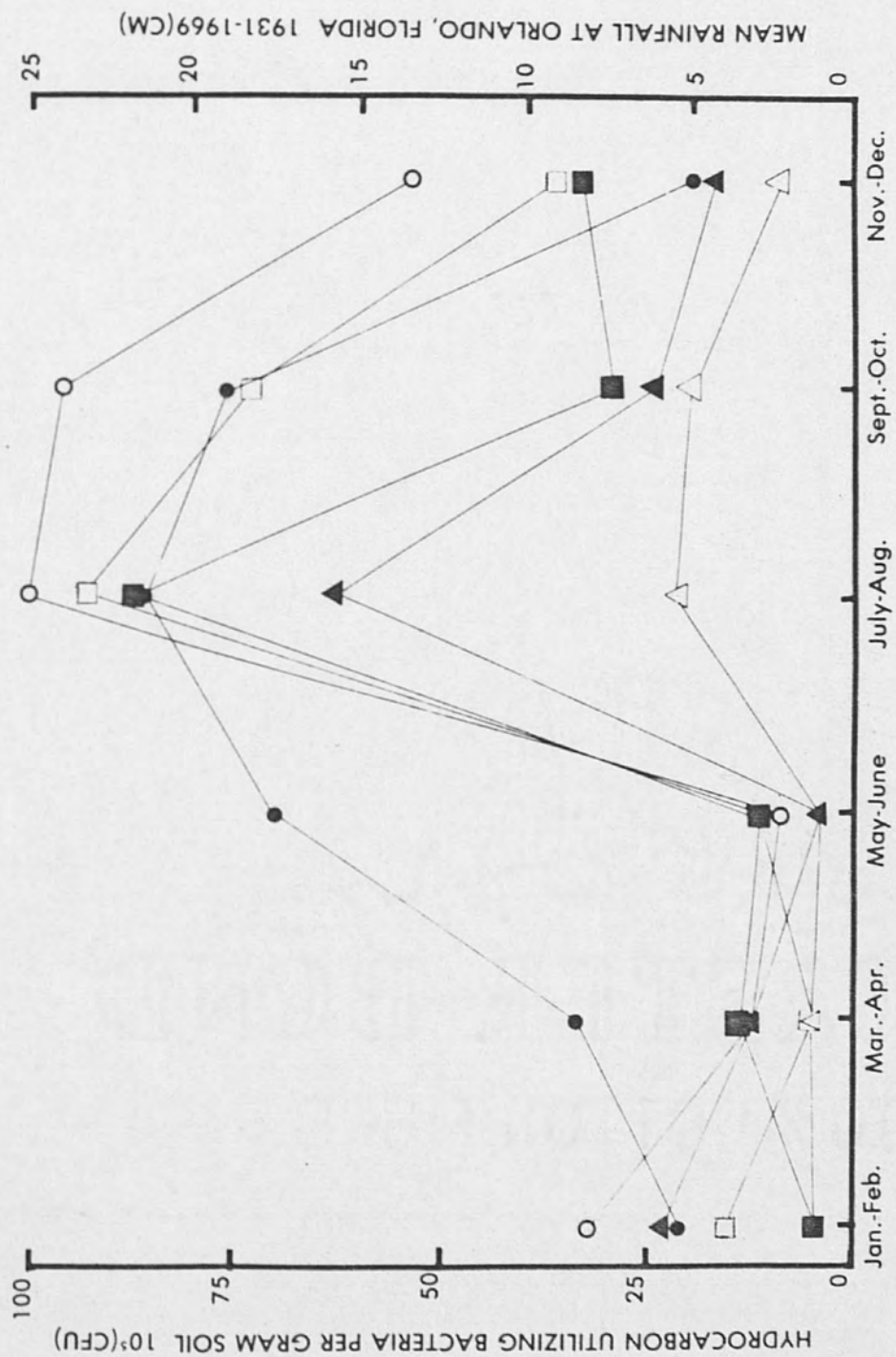
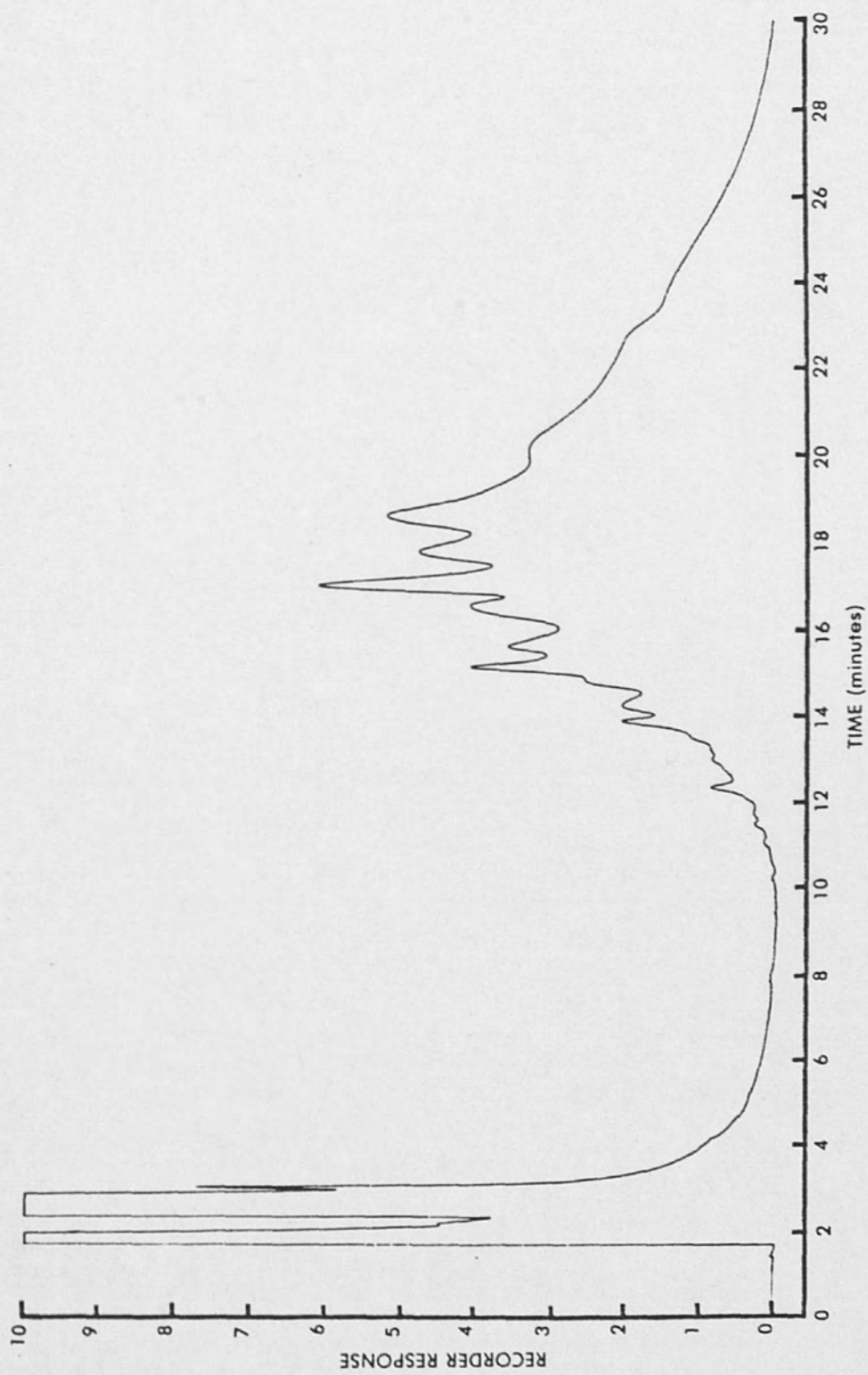


FIG. 3. Gas-liquid chromatographic tracing of the  
chloroform extractable hydrocarbons present in one gram of  
roadside soil (attenuation  $64 \times 10^2$ ).





28

FIG. 4. Gas-liquid chromatographic tracing of the  
chloroform extractable hydrocarbons present in one liter of  
highway stormwater runoff (attenuation 40).

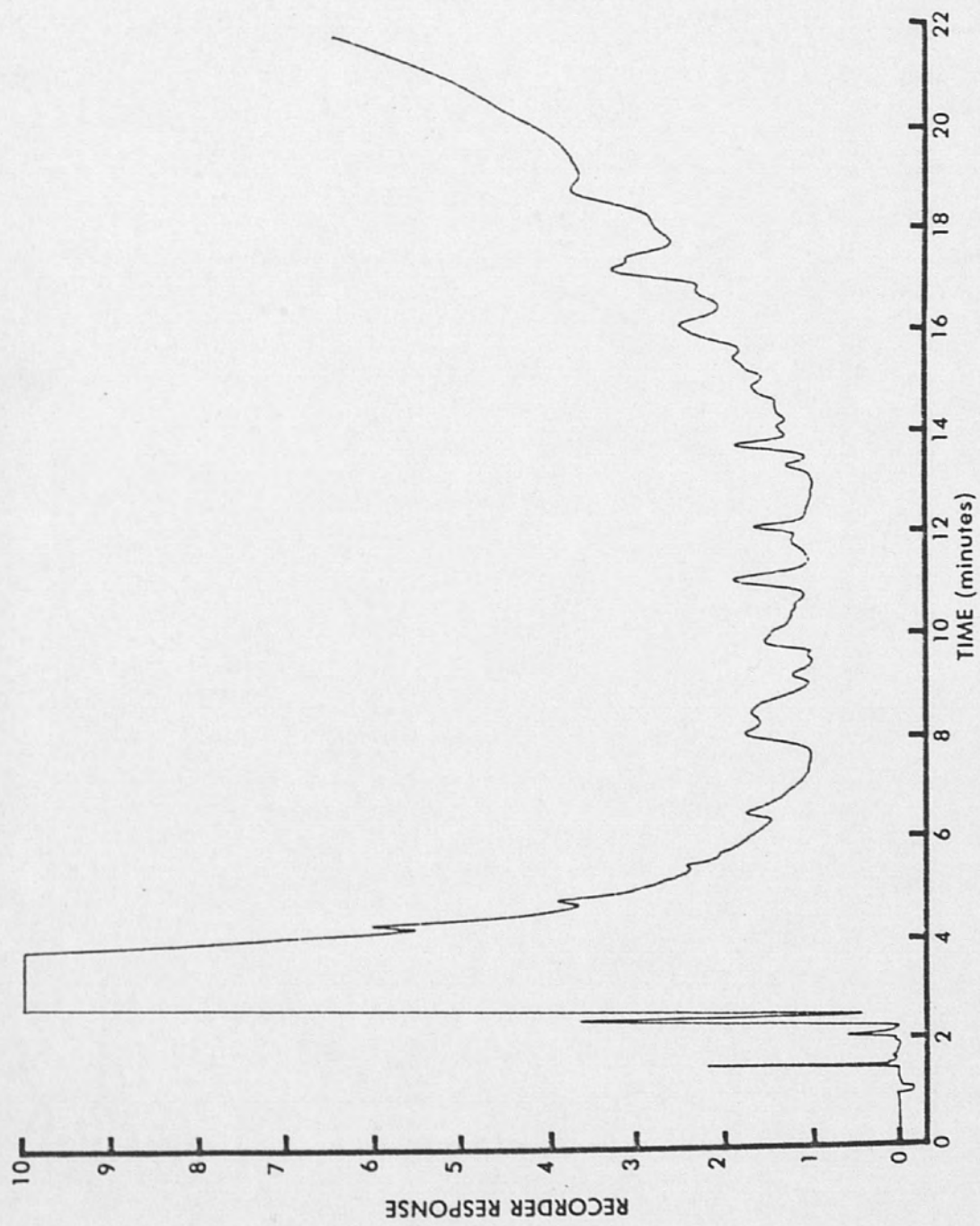


FIG 5. Gas-liquid chromatographic tracings of  
weathered and biodegraded kerosene (attenuation  $64 \times 10^3$ ).



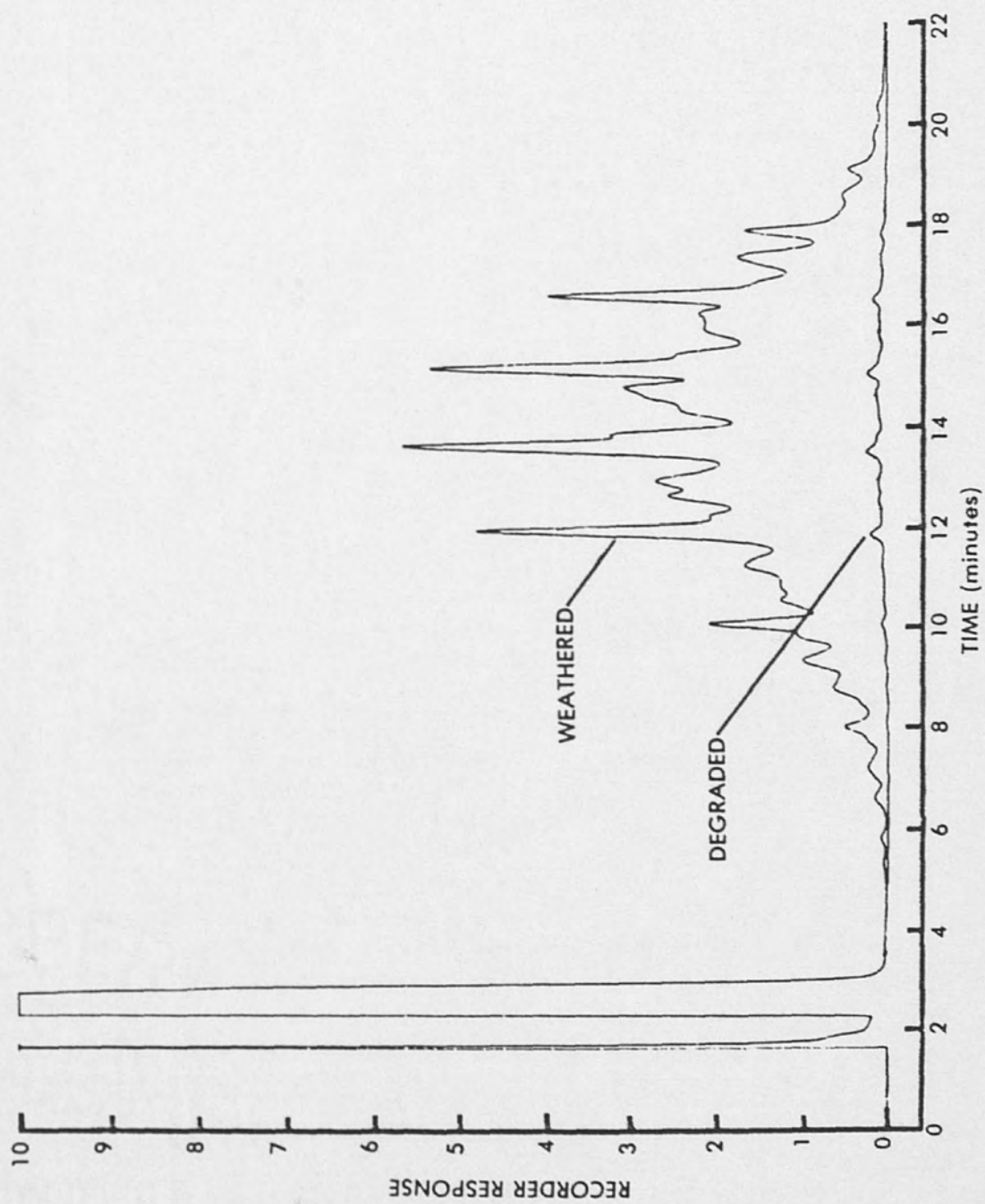


TABLE 4. Concentration of chloroform extractable  
hydrocarbons in roadside soil<sup>a</sup>

---

Distance from the highway pavement (meters)	Concentration of <sup>b</sup> hydrocarbons mg/L <sup>-</sup>
0	0.90
3	0.53
6 <sup>c</sup>	0.21
9	0.23
12	0.15

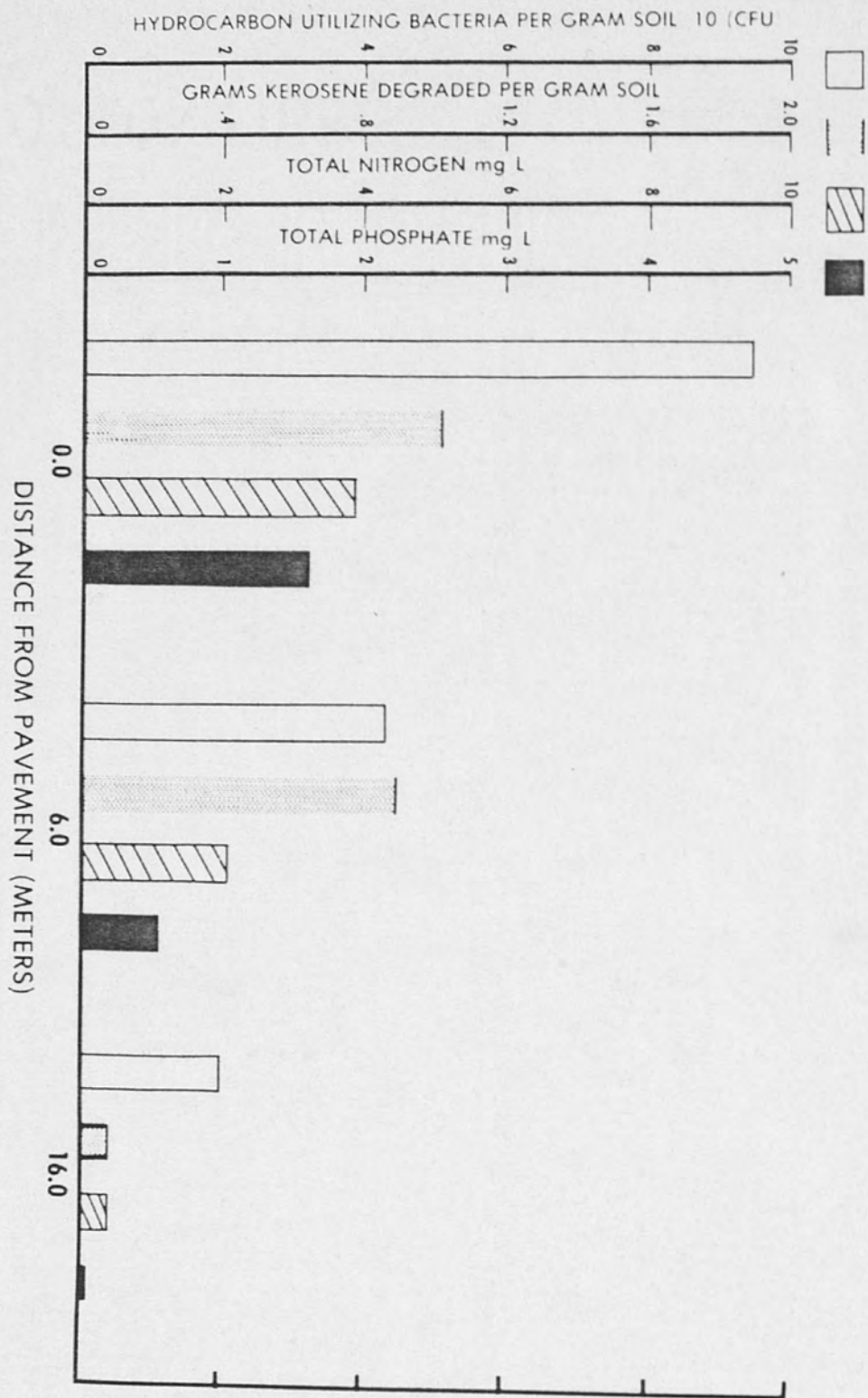
---

<sup>a</sup> Top soil (2.5 cm) of dry soil at the dry site.

<sup>b</sup> Values are averages of triplicate determinations.

<sup>c</sup> Lowest elevation in roadside area.

FIG. 6. The effect of distance from the pavement, bacterial concentration, total nitrogen concentration, and phosphate concentration on the biodegradation of kerosene by soil collected from the dry site during the dry season (January-February).





One gram of roadside soil immediately adjacent to the pavement degraded 50% (1.0 g) of the kerosene under optimum conditions in 60 days (Fig. 6). Bacteria from soil 16.0 m from the pavement were less effective and degraded only 4.0% (0.08 g) of the kerosene in 60 days.

The ability of soil at the edge of the highway to degrade kerosene increased by 75.0% during the rainy months of July and August (Fig. 7). The highest hydrocarbon degradation was observed in soil collected from the edge and 6.0 m distance from the pavement under wet conditions (Fig. 7). One gram of surface soil degraded 87.5% (1.75 g) of the kerosene in 60 days under optimum conditions. The chromatographic tracing of degraded kerosene indicated that all hydrocarbon components were equally susceptible to microbial utilization (Fig. 5).

Inorganic nutrient concentration. The concentrations of nitrogen and phosphate in soil at the edge of the pavement decreased during the rainy season (Fig. 6 and 7). Nitrogen concentrations in soil 6.0 m from the pavement did not change significantly with seasonal variation (Fig. 6 and 7). The highest concentration of nitrogen and phosphate was observed in the water and sediment of the wet site ditch (Fig. 8).

The degradation of kerosene by bacteria present in the surface water of the wet site ditch was 86.0% (1.72 g) in 60 days under optimum conditions (Fig. 8). Anaerobic wet

04

FIG. 7. The effect of distance from the pavement,  
bacterial concentration, total nitrogen concentration, and  
phosphate concentration on the biodegradation of kerosene  
by soil collected from the dry site during the rainy season  
(July-August).

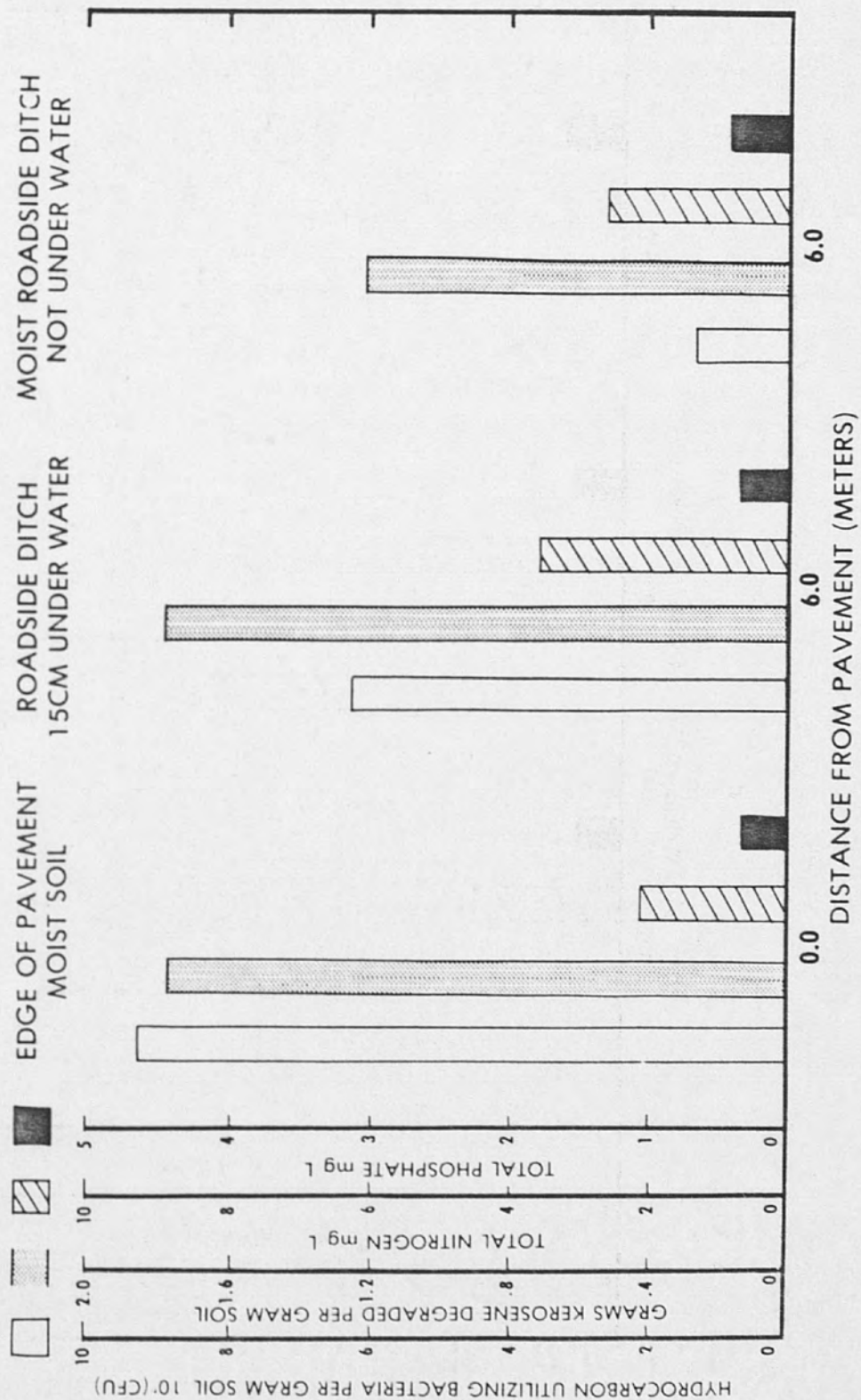
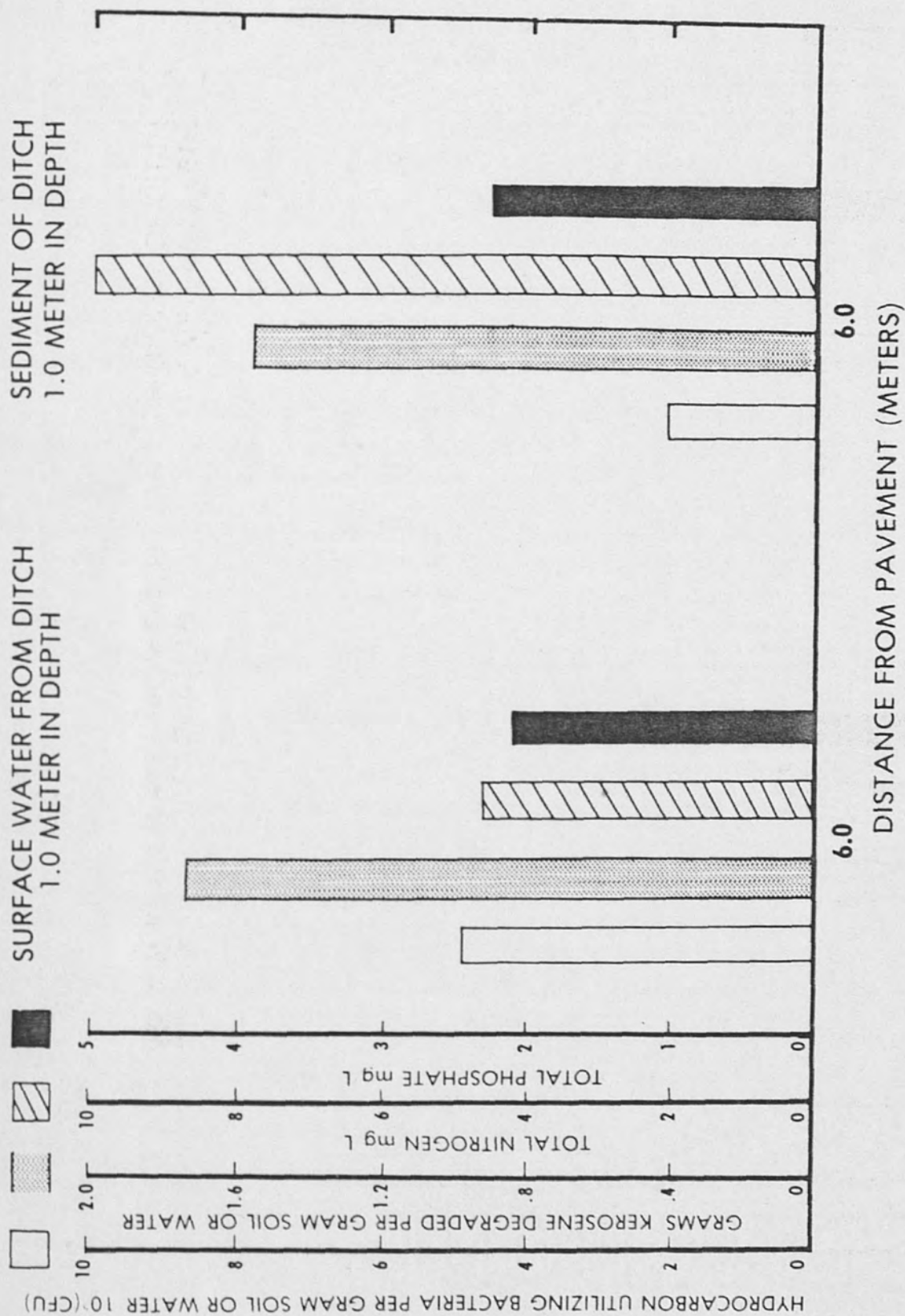


FIG. 8. The effect of distance from the pavement,  
bacterial concentration, total nitrogen concentration, and  
phosphate concentration on the biodegradation of kerosene by  
surface water (top 2.5 cm) and sediment collected from the  
wet site ditch.





ditch sediment samples degraded 77.5% (1.55 g) of the kerosene in 60 days under aerobic conditions (Fig. 8).

The concentration of petroleum utilizing bacteria present in the anaerobic sediment of the wet site was similar to roadside soil 16.0 m from the pavement.

Pseudomonas sp. were the predominant petroleum utilizing bacteria isolated from each roadside soil and water sample (Table 5). Higher species diversity was observed in both the wet soil and water samples (Table 5).

Evaluation of the biodegradation potential. Evaluation of the hydrocarbon degradation potential of roadside soils resulted in the following relative effectiveness: the highest degradation occurred in the samples of sediments from shallow roadside ditches and wet soil at the edge of the highway pavement. Surface water and sediments of anaerobic roadside ditches incubated under aerobic conditions proved less effective. Dry roadside soil was least effective in degrading the hydrocarbons present in storm-water runoff.

TABLE 5. Predominant hydrocarbon bacteria isolated from the roadside environment

Type of environment and conditions	Distance from highway pavement (meters)	Predominant bacteria isolated on kerosene basal salts media
Dry soil (top 2.5 cm)	0	<u>P. aeruginosa</u> , <u>Pseudomonas</u> sp.
Dry soil (top 2.5 cm)	6	<u>Pseudomonas</u> sp., <u>Micrococcus</u> sp.
Dry soil (top 2.5 cm)	16	<u>Pseudomonas alcaligenes</u>
Wet soil (top 2.5 cm)	0	<u>P. alcaligenes</u> , <u>P. putida</u>
Sediment (under 12 cm water)	6	<u>P. pseudocalcaligenes</u> , <u>P. fluorescens</u> , <u>Flavobacterium</u> sp., <u>Pseudomonas</u> sp.
Wet sediment not under water	6	<u>P. testosteroni</u> , <u>P. alcaligenes</u> , 2 <u>Pseudomonas</u> sp.
Surface water of ditch 1.0 m deep	6	<u>P. aeruginosa</u> , <u>Acinetobacter</u> sp., <u>Micrococcus</u> sp.
Sediment of ditch 1.0 m deep	6	<u>P. aeruginosa</u> , <u>Pseudomonas</u> sp.

### Discussion

Bacterial degradation of hydrocarbons is dependent upon environmental conditions: temperature, pH, moisture, nutrient concentration, and redox potential. These conditions are usually not at optimum levels in petroleum polluted environments. However, valuable information on the biodegradation of petroleum can be obtained from laboratory studies in which these environmental conditions are maintained at optimum levels (30). In this study, samples collected from roadside environments were examined for their ability to degrade kerosene under optimum environmental conditions. The results suggested that it is possible to predict the petroleum degradation potential of various roadside environments.

The hydrocarbon degradation potential was dependent upon the species diversity and concentration of petroleum-utilizing microorganisms. Increased bacterial concentrations in roadside samples were directly related to hydrocarbon concentration, inorganic nutrients, and soil moisture.

In petroleum contaminated environments, bacterial populations which are capable of utilizing the hydrocarbons are selected. The subsequent concentration increase has been



well documented in studies of oil contaminated soil and aquatic systems (11,12,19). Zobell (35) observed bacterial concentration increases from  $10^3$ /g to  $10^6$ /g in environments exposed to petroleum. The concentrations of petroleum utilizing bacteria in soils directly exposed to vehicular petroleum waste were two orders of magnitude higher than soils not exposed to these wastes. Increased bacterial concentrations were at a maximum during months of increased rainfall when stormwater volumes increased.

Inorganic nutrient limitation has been observed in soil and aquatic environments exposed to petroleum (2). The addition of inorganic nitrogen and phosphorus to oil contaminated soil has been shown to stimulate the growth of microbial populations and enhance petroleum biodegradation (13,19). Shaheen (24) analyzed the stormwater runoff of highways and observed inorganic nitrogen and phosphorus concentrations of 0.16 g/liter and 1.1 g/liter respectively. The addition of these inorganic nutrients into soils by highway runoff resulted in increased bacterial concentrations.

Increases in the moisture content of soils stimulate bacterial activity and growth. Kincannon (12) studied the degradation of waste oil in soils and demonstrated higher bacterial concentration in soils of increased moisture. In our study the highest degree of kerosene degradation was associated with bacteria from wet roadside soils.

Dry soils were less effective due to decreased solubilization of nutrients which resulted in decreased bacterial activity.

This study demonstrated that bacteria present in roadside environments possess the potential to degrade vehicular waste petroleum. However, optimum environmental conditions seldom occur in roadside soils. Anaerobic sediments which appear capable of degrading hydrocarbons as efficiently as aerobic soil 6.0 m from the pavement are indeed very inefficient. Active biodegradation of hydrocarbons is an oxidative process and requires an aerobic environment (2). Such conditions are non-existent in sediments of deep roadside ditches. Petroleum degradation under these anaerobic conditions is an extremely slow process (2).

The design of roadside ditches and drainage systems influences the degradation of vehicular waste petroleum. Since contamination of adjacent aquatic systems by waste petroleum present in highway runoff has been reported (26), it should be confined to the roadside environment. Pollution abatement should be augmented by microbial degradative processes. Biodegradation can be enhanced by the construction of shallow roadside ditches in which storm-water infiltrates surface soil. Deep ditches, which support anaerobic sediments where hydrocarbons accumulate, should be avoided.

The roadside environment is a unique system of petroleum biodegradation. Factors which severely limit petroleum biodegradation in other environments are less severe. Limiting factors of nutrient concentration, soil moisture, and excessive hydrocarbon loading are alleviated by storm runoff. The combined effects establish a soil microflora capable of degrading a wide range of vehicular related hydrocarbons.

SECTION II: ESTIMATION OF THE OPTIMUM AND IN SITU HYDRO-CARBON DEGRADATION RATES IN THE ROADSIDE ENVIRONMENT

Hydrocarbon mineralization rates were estimated in roadside environments exposed to vehicular waste petroleum. Soil and water samples were incubated under optimum and in situ environmental conditions. Hydrocarbon degradation rates were determined by oxidation of [1-<sup>14</sup>C] hexadecane to <sup>14</sup>CO<sub>2</sub>. Biodegradation rates were 25 to 126-fold higher under optimum environmental conditions as compared to in situ rates. The highest in situ hydrocarbon degradation rate was observed in wet soils immediately adjacent to the highway pavement (92.1 µg/g soil h.<sup>-1</sup>). The relative biodegradability of hexadecane and kerosene was compared. Both were equally susceptible to degradation by microorganisms in the roadside environment. Hydrocarbon degradation rates were compared to those observed in other petroleum contaminated environments.



## Introduction

Several studies have been reported which estimate the in situ biodegradation rates of petroleum contaminated environments (22,23,30). However, most have been concerned with marine and aquatic environments. Few studies have been published which estimate the in situ hydrocarbon degradation rates in soil exposed to vehicular waste petroleum.

The purpose of this investigation was to estimate the optimum and in situ biodegradation rates of vehicular waste hydrocarbons in soil and aquatic environments adjacent to highways.

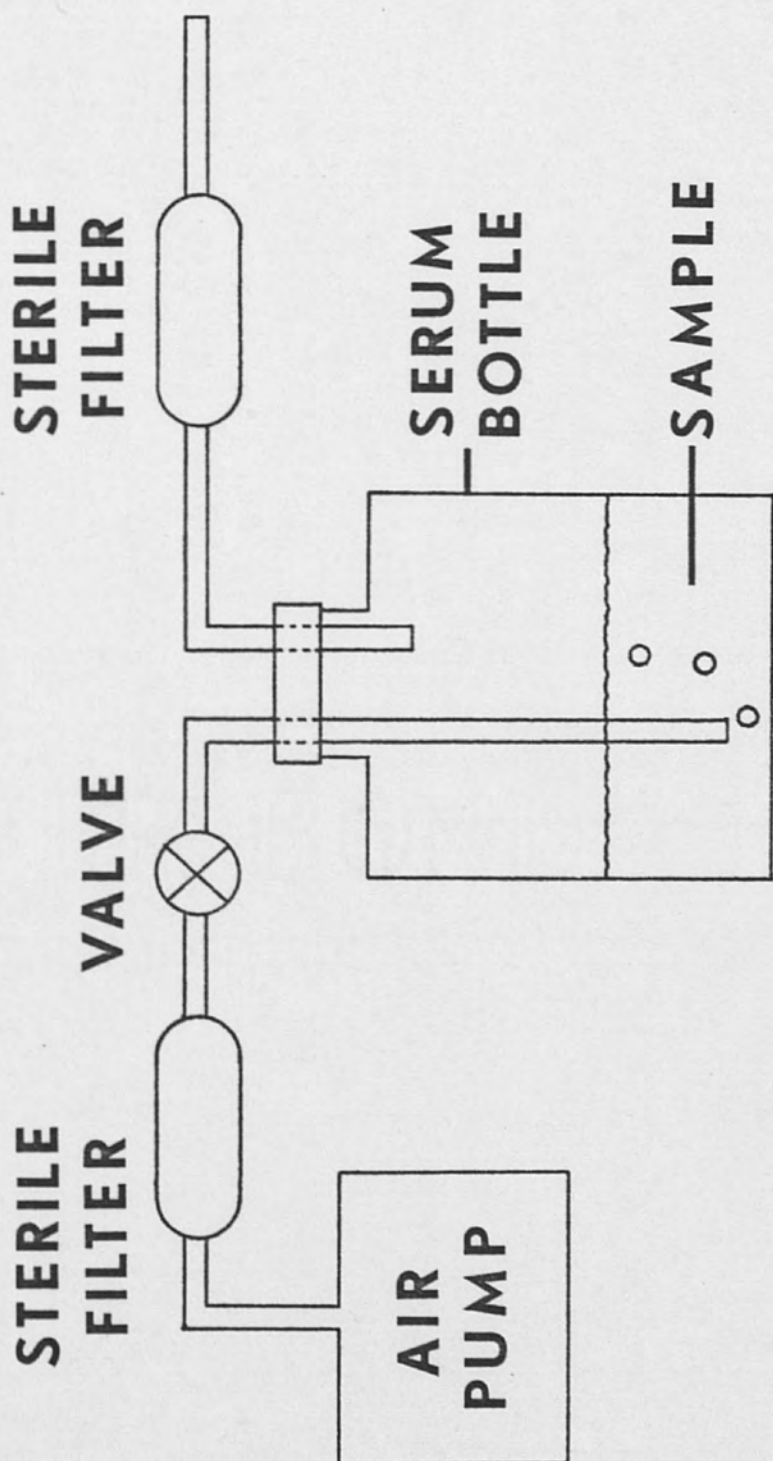
The results indicated that the microorganisms present in the roadside environment were capable of degrading hydrocarbons representative of those present in highway stormwater runoff. Optimum rates of biodegradation indicated the relative effectiveness of roadside samples, but did not reflect actual in situ rates. Hydrocarbon biodegradation rates were highest in wet soils directly exposed to waste petroleum. Lower rates of degradation were observed in dry soils and in soils not exposed to highway runoff. Hydrocarbon degradation by anaerobic sediments of wet drainage ditches was insignificant when incubated under in situ anaerobic conditions.

## Materials and Methods

Sample sites and methods. Composite soil and water samples were collected from the wet and dry roadside environments as previously described.

Hexadecane-kerosene assay. The relative biodegradability of hexadecane and kerosene was determined by inoculating hydrocarbon basal salts solution with roadside surface soil and water samples. Sterile serum bottles (60 ml) containing 30 ml sterile basal salts solution as described previously were inoculated with composite roadside samples (1.0 g or 1.0 ml). Sterile hexadecane (0.40 ml, 0.50 g) were added to each bottle. Sterile controls were utilized to determine weathering and volatilization effects. Each serum bottle was assembled with a sterile air sparger constructed of glass tubing and sterile cotton air filters (Fig. 9). The environmental samples were continuously aerated for 70 h. at a rate of 50 ml/min. Samples were run in replicates of four. After 70 h., the contents were extracted with chloroform and quantitatively determined by gas-liquid chromatography as previously described. The data were corrected for volatilization and weathering effects. The results were expressed as percent hexadecane

FIG. 9. An apparatus for determining the optimum relative biodegradability of a hexadecane-kerosene mixture.





and kerosene degraded.

Samples collected from anaerobic sediments of deep roadside ditches were placed in basal salts solution and incubated in an aerobic glove box chamber (Coy Co.). After 70 h. incubation, the samples were removed from the chamber and extracted with chloroform as previously described.

Optimum rates determination. Optimum hydrocarbon degradation rates were estimated by inoculating roadside soil and water samples into basal salts with  $[1-^{14}\text{C}]$  hexadecane and measuring  $^{14}\text{CO}_2$  evolution during a 70 h. incubation. Aerobic roadside samples (1.0 g or 1.0 ml) were incubated at  $23^\circ\text{C}$  in serum bottles (60 ml) containing 30 ml basal salts solution. The serum bottles were equipped with a  $^{14}\text{CO}_2$  trapping apparatus as described by Smith (25). Twenty-five  $\mu\text{l}$  of  $[1-^{14}\text{C}]$  hexadecane (Amersham-Searle Corp.) diluted with nonradioactive hexadecane (Fisher Scientific Co.) to a specific activity of  $1.2 \times 10^{-3} \mu\text{Ci}/\mu\text{M}$  was added to each sample bottle. The total activity per sample was 0.1  $\mu\text{Ci}$ . The sample bottles were incubated and counted at 10 h. intervals. The total incubation time was 70 h. At the end of the incubation period, 1.0 ml of 10 N  $\text{H}_2\text{SO}_4$  was added to each sample to stop microbial growth and release  $^{14}\text{CO}_2$  from the sample solution. The  $^{14}\text{CO}_2$  was transferred via an airstream from the serum bottle and passed through scintillation vials containing a trapping

solution in a toluene scintillation fluor (25). The air stream was allowed to flow for 5 min. Each scintillation vial contained 10 ml of Omniscint (I.C.N. Co.) scintillation fluor in toluene (4.0 g/liter), plus 2.5 ml of the  $^{14}\text{CO}_2$  trapping solution as described by Smith (25).

The scintillation vials were counted in triplicate on a Tricarb Liquid Scintillation Spectrometer (Packard Instrument Co.) with a 6% gain and a 50-1000 window setting. Corrections for background were made on all samples. Counting efficiency was determined to be 82% by the internal standard method (32).

The rates of  $[1-^{14}\text{C}]$  hexadecane degradation were derived from the linear portion of exponential plots of percent  $^{14}\text{CO}_2$  evolution with time. The optical density of each sample was monitored to establish the exponential growth interval. Degradation rate calculations were based on the assumption that 100%  $^{14}\text{CO}_2$  evolution reflected microbial utilization of  $8.54 \times 10^{-2}$  mM hexadecane.

Anaerobic sediment samples were incubated in an anaerobic chamber. The reactions were quenched with 1.0 ml of 10 N  $\text{H}_2\text{SO}_4$  at 10 h. intervals, removed from the chamber and assayed as described above.

In situ rate determination. In situ rates of hydrocarbon oxidation were estimated by the methods previously described, except that the environmental samples were not enriched with nutrient salts. Twenty-five  $\mu\text{l}$   $[1-^{14}\text{C}]$

hexadecane (0.1  $\mu$ Ci) and 5.0 ml sterile distilled water was added to each (1.0 g or 1.0 ml) roadside sample.

## Results

### Comparison of hexadecane-kerosene biodegradability.

The microorganisms present in each roadside sample degraded kerosene as effectively as hexadecane when incubated under optimum environmental conditions ( $r = 0.82$ ,  $p < 0.05$ , Table 6). The chromatographic tracings of sterile weathered and degraded hexadecane-kerosene indicated that all hydrocarbons were equally susceptible to microbial utilization (Fig. 10 and 11).

The highest degree of hydrocarbon degradation occurred in the wet sediment of a shallow drainage ditch 6.0 m from the pavement, and in the surface soil immediately adjacent to the highway pavement (Table 6). Degradation was less efficient in the dry soil and the amount of hydrocarbons degraded in 70 h. decreased with distance from the pavement ( $r = -0.70$ ,  $p < 0.05$ , Table 6). Hydrocarbon degradation by anaerobic sediment incubated under anaerobic conditions for 70 h. was insignificant.

Optimum [1-<sup>14</sup>C] hexadecane degradation rates. The optimum rates of hydrocarbon degradation by microorganisms present in dry roadside soils increased 87% (8.9 to 16.7  $\mu\text{M/h.}$ ) under wet conditions (Table 7). The highest



TABLE 6. Comparison of kerosene and hexadecane biodegradation under optimum conditions

Roadside environment	Distance from highway pavement (meters)	Hydrocarbon degraded <sup>a</sup>			
		Kerosene		Hexadecane	
		% <sup>c</sup>	grams	% <sup>c</sup>	grams mM
Dry soil (top 2.5 cm)	0	87.2	0.38	82.0	0.37 1.65
Dry soil (top 2.5 cm)	16	65.9	0.27	41.1	0.16 0.72
Dry soil (top 2.5 cm)	60	61.7	0.25	28.2	0.09 0.42
Wet soil (top 2.5 cm)	0	95.7	0.42	92.3	0.43 1.89
Wet soil (top 2.5 cm)	6	74.4	0.31	87.2	0.40 1.77
Sediment under 12 cm water	6	95.7	0.42	93.0	0.43 1.90
Surface water	6	78.7	0.33	95.4	0.44 1.96
Sediment under 1 m water <sup>b</sup>	6	0.0	0.0	0.0	0.0 0.0

<sup>a</sup> 70 h. incubation in nutrient salts and aerated 50 cc per min.

<sup>b</sup> Incubation under anaerobic conditions.

<sup>c</sup> Percent degradation corrected with sterile weathered controls.

0a

FIG. 10. Gas-liquid chromatographic tracing of a sterile weathered hexadecane-kerosene mixture following 70 h. incubation (attenuation  $16 \times 10^3$ ).

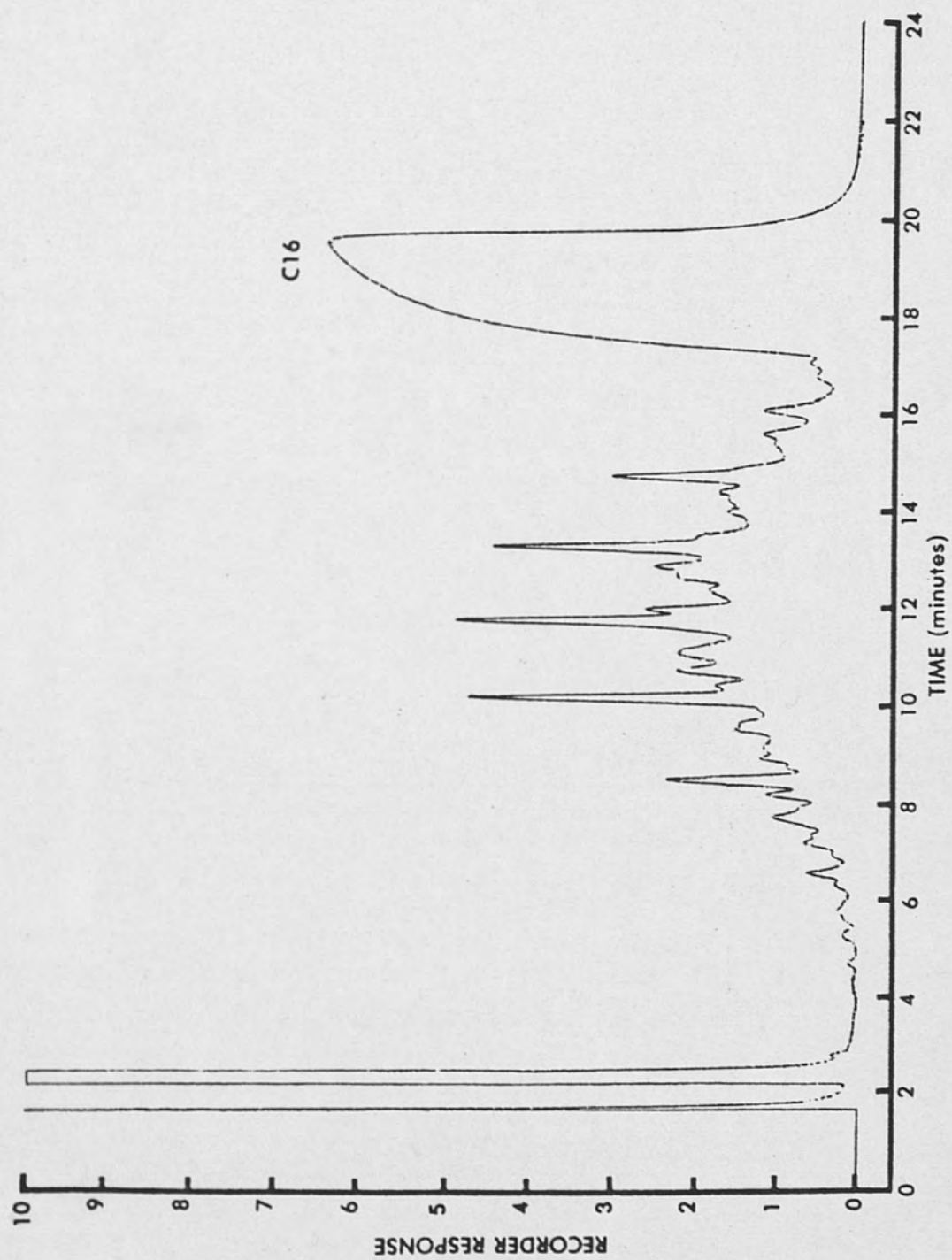


FIG. 11. Gas-liquid chromatographic tracing of bio-  
degraded hexadecane-kerosene mixture following 70 h.  
incubation (attenuation  $16 \times 10^3$ ).



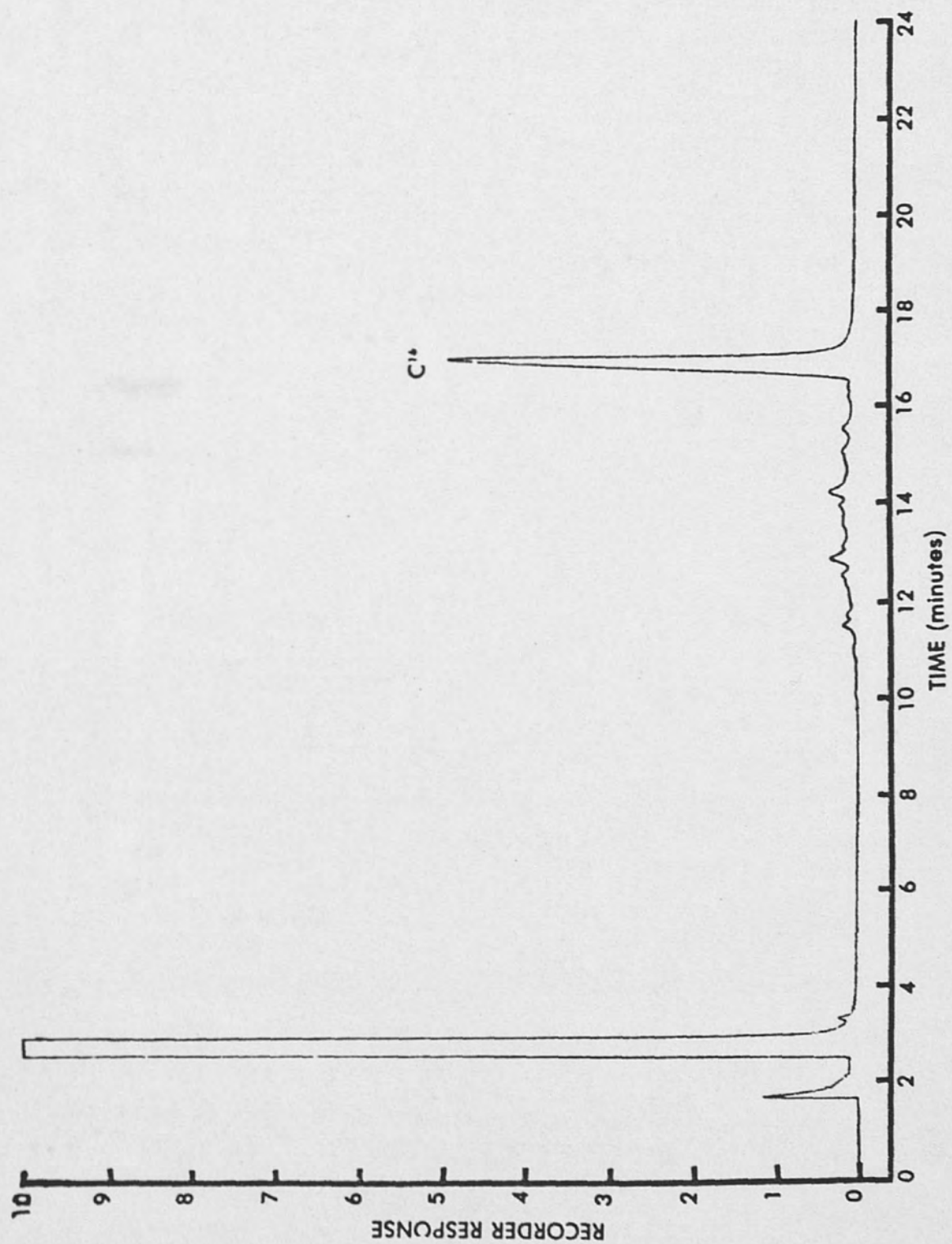


TABLE 7. Comparison of hydrocarbon biodegradation under optimum and in situ conditions

Roadside environment	Distance from highway pavement (meters)	Slope log $\Delta$ od vs time	<sup>a</sup> $\mu$ M hexadecane degraded		
			Optimum <sup>b</sup> $\frac{-1}{\mu\text{M g h.}}-1\text{d}$	In situ <sup>c</sup> $\frac{-1}{\mu\text{M g h.}}-1\text{d}$	70 h.
Dry soil (top 2.5 cm)	0	0.13	8.9	0.14	10.4
Dry soil (top 2.5 cm)	16	0.06	7.0	0.13	9.4
Dry soil (top 2.5 cm)	60	0.03	2.1	0.08	7.0
Wet soil (top 2.5 cm)	0	0.16	16.7	0.41	29.5
Wet soil (top 2.5 cm)	6	0.13	14.5	0.21	15.6
Sediment under 12 cm water	6	0.12	13.1	0.20	12.2
Surface water of ditch	6	0.26	16.6	0.13	9.9
Sediment under 1 m water <sup>e</sup>	6	0.0	0.0	0.0	0.0

<sup>a</sup> Calculated from percent  $^{14}\text{CO}_2$  evolved.<sup>b</sup> Nutrient salts added.<sup>c</sup> Sample plus 2.0 ml sterile distilled water.<sup>d</sup> Calculated from slope of exponential growth curves.<sup>e</sup> Incubation under anaerobic conditions.

hydrocarbon degradation rate ( $16.7 \mu\text{M hexadecane g}^{-1} \text{ soil h}^{-1}$ ) was observed in the wet surface soils immediately adjacent to the highway pavement. Hexadecane degradation rates in wet roadside samples were two-fold higher than in dry soil samples (Table 7).

A positive correlation was observed between the rates of hexadecane oxidation and the increase in optical density of each sample ( $r = 0.85$ ,  $p < 0.05$ , Table 7).

Comparison of optimum and in situ degradation. The rates of hexadecane oxidation in samples incubated under in situ environmental conditions were 25 to 126-fold less than samples supplemented with nutrient salts (Table 7). The largest difference between nutrient supplementation and in situ incubation was observed in the surface water samples.

The relative degradative effectiveness of roadside samples incubated under in situ environmental conditions was similar to that observed in the optimum rate study. In situ degradation rates were highest in wet soils and sediment directly exposed to stormwater runoff. In situ hexadecane degradation rates decreased with distance from the highway pavement ( $r = -0.99$ ,  $p < 0.01$ ).

The oxidation of hexadecane by microorganisms present in the environment was enhanced by nutrient supplementation and aeration. Samples aerated in nutrient salts were capable of degrading twice the amount of hexadecane as were non-aerated samples supplemented with nutrient salts (Tables 6

and 7). Roadside samples incubated under optimum conditions with the addition of nutrients and dissolved oxygen did not reflect the actual in situ rates of biodegradation.



### Discussion

Several studies have been done to determine the relative biodegradation potential of various petroleum-polluted environments (6,21,30,31). These studies provide valuable information concerning the biodegradation of petroleum pollutants, but do not offer a true indication of biodegradation rates under in situ conditions. Knowledge of these biodegradation rates is essential for determining the actual potential of an environment for degradation. Furthermore, practical applications based on rate studies can be implemented to enhance degradation and thus abate petroleum pollution.

In this study, rates of hydrocarbon degradation were estimated in roadside environments exposed to vehicular waste petroleum. Based on these rate studies, suggestions can be made to design roadside environments to enhance the hydrocarbon biodegradative processes.

The oxidation rates of radiolabeled hydrocarbons served as the basis for estimating the degradation rates of vehicular waste petroleum. The use of radiolabeled hydrocarbons is the most widely accepted method for determining petroleum degradation rates in polluted environments (2).

Since complex mixtures of radiolabeled hydrocarbons are not commercially available or are very expensive, several biodegradation studies have utilized  $[1-^{14}\text{C}]$  hexadecane as a hydrocarbon representative of petroleum pollutants (7,23,30). However, it has been reported that normal alkanes are among the least recalcitrant hydrocarbons present in refined petroleum (13,18), hence biodegradation rates of hexadecane may not reflect the mineralization rates of vehicular waste petroleum. To determine the feasibility of substituting hexadecane for vehicular waste petroleum hydrocarbons, the biodegradability of hexadecane and a complex mixture of aromatic and aliphatic hydrocarbons (kerosene) was compared. Kerosene contains all classes of hydrocarbons present in vehicular waste petroleum and has been implemented as a model hydrocarbon mixture for refined petroleum biodegradation studies (5). The results of the biodegradability comparison indicated that a positive correlation exists between the biodegradability of kerosene and hexadecane. The hydrocarbon components of kerosene and the hexadecane were equally susceptible to microbial utilization by the roadside microflora. Thus,  $[1-^{14}\text{C}]$  hexadecane was utilized as the representative hydrocarbon for this investigation.

Several studies have estimated hydrocarbon degradation rates by incubating environmental samples under artificially optimized conditions of nutrient enrichment (7,22,23).

Inorganic nutrient concentrations are reduced in petroleum contaminated environments and become a major biodegradation rate limiting factor. The biodegradation rates of petroleum are enhanced by the addition of inorganic nitrogen and phosphorus (2,13). Thus, studies in which environmental samples are incubated under optimal conditions of nutrient enrichment evaluate biodegradation potentials but do not accurately assess in situ rates of hydrocarbon mineralization.

This investigation was designed to evaluate both biodegradation potential and actual in situ rates of vehicular waste petroleum. Roadside environmental samples were incubated under both optimum and in situ environmental conditions. Samples supplemented with inorganic nutrient salts degraded [1-<sup>14</sup>C] hexadecane at rates 25 to 126-fold higher than the rates observed under in situ environmental conditions.

Both optimum and in situ rates of [1-<sup>14</sup>C] hexadecane degradation were highest in the roadside soil in close proximity to the highway and in the shallow drainage ditches. Degradation rates decreased in soils as distance from the pollution source increased.

Active hydrocarbon degradation is an oxidative process and occurs at extremely slow rates in anaerobic environments (35). Hydrocarbon degradation in an anaerobic aquatic environment was observed to occur at undetectable rates using radiolabeled [1-<sup>14</sup>C] hexadecane (2).

In this study, oxidation of  $[1-^{14}\text{C}]$  hexadecane by micro-organisms present in the sediments of deep roadside ditches was also undetectable during a 70 h. anaerobic incubation.

In situ hydrocarbon degradation rates have been estimated in petroleum contaminated environments. Seki (23) studied hexadecane degradation in Tokyo Bay and reported  $0.015 \text{ g degradation / m}^3 \text{ seawater day}^{-1}$ . Walker and Colwell (30) reported  $0.05 \text{ g hexadecane degradation / m}^3 \text{ water day}^{-1}$  in an oil polluted estuary. Hexadecane degradation in the roadside ditch surface water was estimated at  $0.13 \text{ } \mu\text{M ml}^{-1} \text{ h}^{-1}$  or  $706 \text{ g / m}^3 \text{ surface ditch water day}^{-1}$ .

The higher rates of hexadecane oxidation observed in roadside samples can be attributed to nutrient supplementation by nitrogen and phosphorus present in stormwater runoff. Aquatic samples incubated under artificially optimized conditions have hydrocarbon degradation rate increases of  $25\text{--}2500 \text{ g / m}^3 \text{ day}^{-1}$  (2).

Few studies have estimated in situ hydrocarbon degradation rates in soil (12,13,19). These include the application of large volumes of waste oil to soil and measure percent reduction with time. In such studies degradation measurements are difficult to determine due to volatilization and migration of oil through surface soil. Kincannon (12) estimated biodegradation rates in surface soil supplemented with inorganic fertilizers. Refinery waste oil was



applied and degradation was observed at  $1 \text{ lb./ft.}^3 \text{ mo.}^{-1}$  or  $534 \text{ g/m}^3$  surface soil  $\text{day}^{-1}$ . In the present investigation, the microflora of the roadside soil degraded hexadecane at a rate of  $0.14 \text{ } \mu\text{M g}^{-1} \text{ soil h.}^{-1}$  or  $761 \text{ g/m}^3$  surface soil (top 2.5 cm)  $\text{day}^{-1}$ .

Applying the highway hydrocarbon deposition model of Shaheen (24), it was calculated that the highway pavement adjacent to the roadside environment in this study received waste petroleum at a rate of  $167 \text{ g mile}^{-1} \text{ day}^{-1}$  or  $103.5 \text{ g km}^{-1} \text{ day}^{-1}$ . The rates of hydrocarbon degradation in situ were shown to exceed this hydrocarbon deposition rate. The in situ hydrocarbon degradation rate ( $761 \text{ g/m}^3$ ) of the adjacent roadside surface soil (1 m in width, top 2.5 cm) was  $3.04 \times 10^4 \text{ g mile}^{-1} \text{ day}^{-1}$  or  $1.90 \times 10^4 \text{ g km}^{-1} \text{ day}^{-1}$ . From this study it can be concluded that the indigenous microflora of the roadside ecosystem are capable of removing the vehicular waste petroleum. In roadside environments which receive higher hydrocarbon deposition due to increased traffic volume, roadside environments should be constructed to enhance hydrocarbon degradation. Such design should include the construction of shallow drainage ditches and holding ponds which would support active aerobic microbial degradation.

## GENERAL DISCUSSION

Aquatic and terrestrial roadside environments are exposed to hydrocarbons of vehicular origin. Soils adjacent to the highway pavements in this study contained concentrations of extractable hydrocarbons ranging from 0.9 to 1.5 mg/L (Table 4). Gas-liquid analyses of extracted highway stormwater indicated the presence of refined petroleum hydrocarbons (Fig. 4).

The addition of vehicular waste petroleum to adjacent roadside soil had a significant impact upon the concentrations of petroleum utilizing microorganisms. During months of increased rainfall and stormwater runoff, petroleum utilizing bacterial populations increased two-fold (Fig. 2). Species diversity of petroleum utilizing bacteria increased in terrestrial and aquatic environments directly exposed to highway stormwater (Table 3). Increases in species diversity and microbial concentration indicated the presence of an indigenous roadside microflora capable of degrading a wide variety of hydrocarbon classes. Mixed cultures of petroleum utilizing bacteria isolated from roadside environmental samples demonstrated the ability to degrade all hydrocarbon components of kerosene under optimum environmental

conditions (Fig. 5 and 11).

The ability to degrade hydrocarbons differs among environmental samples collected from roadsides of different design (Table 6). This data suggests that the design of roadside easements and drainage systems play an important role in determining the rate of vehicular waste petroleum mineralization. Surface soils in close proximity to the highway pavement and shallow drainage ditch systems have the highest potential for petroleum biodegradation (Table 7). Roadside drainage systems which support anaerobic sediments are least effective in enhancing biodegradation. The data suggests that vehicular hydrocarbon oxidation rates are extremely slow in the sediment of deep drainage ditches and may enhance waste petroleum accumulation (Table 7).

In the aerobic roadside environments of this study, the lowest in situ biodegradation rates ( $761 \text{ g/m}^3$  surface soil, top  $2.5 \text{ cm day}^{-1}$ ) exceed the estimated hydrocarbon deposition rate ( $4.14 \text{ g/m}^3 \text{ day}^{-1}$  adjacent roadside surface soil) 190-fold. This data suggests that roadside environments designed to enhance aerobic biodegradation are very efficient in the mineralization of vehicular waste petroleum.

From the data presented in Sections I. and II., a roadside drainage ditch design system to enhance vehicular waste oil biodegradation can be suggested.

Highway surfaces should be designed to remove stormwater

from the pavement surfaces rapidly. This enhances removal of highway surface contaminants. The stormwater should be discharged into road easements or shallow drainage ditches where waste petroleum can infiltrate surface soil and remain in an aerobic environment. Highway related petroleum pollutants should be confined to roadside environments for biodegradation and not permitted to contaminate other aquatic ecosystems. Deep drainage systems which augment the formation of anaerobic sediments should be avoided. In highways where adjacent aerobic roadside environments are not feasible, stormwater runoff should be channeled through concrete pipes or flumes and discharged into aerobic environments.

Future investigations into the environmental impact of vehicular waste petroleum should include additional biodegradation studies. Vehicular waste petroleum, e.g., crank-case oil, could be radiolabeled and fractioned. Such preparations would be very expensive, but when utilized in in situ rate studies would provide the best assessment of roadside biodegradation. The concentration at which nutrient limitation occurs could be evaluated by adding various concentrations of phosphorus and nitrogen to roadside environmental samples and measuring degradation rates. Data from these studies could evaluate different roadside design to determine if inorganic nutrients do indeed become a limiting factor of vehicular waste petroleum biodegradation.



## GENERAL SUMMARY

The roadside environment is the land area between the highway pavement and right-of-way boundary line. Such areas are designed to delineate the highway activities from urban and residential areas, but also serve as a region of highway related pollution confinement. The design of the roadside easement and the adjacent stormwater drainage systems determine the fate of vehicular waste petroleum pollutants. Roadside design can enhance or retard the natural processes of microbial biodegradation.

From the investigations presented in Sections I. and II., several observations were made concerning the roadside environment as an effective means of removing highway petroleum pollutants.

The roadside is indeed exposed to hydrocarbons of vehicular origin. Extractions of soil and water directly exposed to stormwater runoff contained a wide variety of hydrocarbons very similar to refined petroleum products. The concentrations of these hydrocarbons were highest in the roadside areas directly exposed to stormwater runoff. Hydrocarbon concentrations were shown to decrease with distance from the highway where the vehicular waste petroleum

pollutants were deposited.

The presence of petroleum pollutants in the roadside environment stimulated the proliferation of microorganisms which utilize hydrocarbons for growth. Bacterial concentrations increased during the months of increased rainfall and rates of hydrocarbon degradation were highest during wet soil conditions. Higher bacterial concentrations and increased degradation rates were the direct result of increased hydrocarbon deposition and inorganic nutrient solubilization by stormwater runoff.

The roadside environment was shown to be a unique system of petroleum degradation. An indigenous hydrocarbon utilizing microflora was continually maintained by the deposition of both hydrocarbons and inorganic nutrients. The microbial populations were present in sufficient concentration and diversity to degrade hydrocarbons at rates which exceeded the waste oil deposition.

The findings of both investigations suggest that the roadside hydrocarbon degradation rates can be enhanced by designing highways with roadsides which confine petroleum pollutants and support active aerobic biodegradative processes. Such design should include the construction of shallow drainage systems which rapidly remove stormwater and waste petroleum from the highway surface. The runoff should then enter shallow roadside ditches within the confines of the highway roadside environment where biodegradation can

occur most efficiently.

## LITERATURE CITED

1. American Public Health Association. 1971. Standard methods for the examination of water and wastewater, 13th ed. American Public Health Association, Inc., New York.
2. Bartha, R. and R. M. Atlas. 1977. The microbiology of oil spills. *Adv. Appl. Microbiol.* 22:225-266.
3. Brock, T. D. 1970. Biology of microorganisms, p. 591. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.
4. Buchanan, R. E. 1974. Part 7 gram negative aerobic rods and cocci, p. 217-273. *In* R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams and Wilkins Co., Baltimore.
5. Buckley, E. N., R. B. Jonas, and F. K. Pfaender. 1976. Characterization of microbial isolates from an estuarine ecosystem: relationship of hydrocarbon utilization to ambient hydrocarbon concentrations. *Appl. Environ. Microbiol.* 32:232-237.
6. Calder, J. A. and J. H. Lader. 1976. Effect of dissolved aromatic hydrocarbons on the growth of marine bacteria in batch culture. *Appl. Environ. Microbiol.* 32:95-101.
7. Caparello, D. M. and P. A. LaRock. 1975. A radioisotope assay for the quantification of hydrocarbon biodegradation potential in environmental samples. *Microbial Ecology* 2:28-42.
8. Dagley, S. 1975. A biochemical approach to some problems of environmental pollution, p. 81-138. *In* P. N. Campbell and F. Dickens (ed.), *Essays in Biochemistry*, Vol. 2, Academic Press, Inc. New York.
9. Davis, J. B. 1967. Petroleum microbiology, p. 282-349. American Elsevier Publishing Co., Inc. New York.
10. Hood, M. A., S. W. Bishop, S. P. Mereys, and T. Whelan, III. 1975. Microbial indicators of oil-rich marsh



sediments, Appl. Environ. Microbiol. 30:982-987.

11. Horowitz, A. and R. M. Atlas. 1977. Response of microorganisms to an accidental gasoline spillage in an arctic freshwater ecosystem. Appl. Environ. Microbiol. 33:1252-1258.
12. Kincannon, C. B. 1972. Oily waste disposal by soil cultivation process. U.S. Environmental Protection Agency, Report EPA-R2-72-110, Washington, D.C.
13. Lektomäki, M. and S. Neimelä. 1975. Improving microbial degradation of oil in soil. Ambio 4:126-129.
14. Miller, J. C. 1977. Ground-water pollution problems in the southeastern United States, p. 186-189. U.S. Environmental Protection Agency, Report 600-3-77-012, Washington, D.C.
15. Munzer, S. R., F. P. Grad, and S. L. Schwartz. 1974. Water pollution problems related to the automobile crankcase oil disposal, p. 457-468. In F. P. Grad and A. J. Rosenthal (ed.), The automobile and the regulation of its impact on the environment. University of Oklahoma Press, Norman, Oklahoma.
16. National Academy of Sciences. 1977. Ozone and other photochemical oxidants, p. 13-44. The National Research Council, Washington, D.C.
17. Parker, C. A., M. Freegate, and C. G. Hatchard. 1971. Chemical and biological factors of crude oil at sea, p. 237-244. In P. Hepple (ed.), Water pollution by oil. Elsevier Pub. Co., New York.
18. Perry, J. J. and C. E. Cerniglia. 1973. Studies on the degradation of petroleum by filamentous fungi, p. 89-94. In D. G. Ahearn and S. P. Meyers (ed.), The microbial degradation of oil pollutants. Louisiana State University Publ. no. LSU-SG-73-01, Baton Rouge.
19. Raymond, R. L., J. O. Hudson, and V. W. Jamison. 1976. Oil degradation in soil. Appl. Environ. Microbiol. 31:522-535.
20. Raymond, R. L., V. W. Jamison, and J. O. Hudson. 1967. Microbial hydrocarbon co-oxidation. I. Oxidation of mono- and dicyclic hydrocarbons by soil isolates of the genus Nocardia. Appl. Microbiol. 15:357-365.

21. Roubal, G. and R. M. Atlas. 1978. Distribution of hydrocarbon-utilizing microorganisms and hydrocarbon biodegradation potentials in Alaskan Continental Shelf areas. *Appl. Environ. Microbiol.* 35:897-905.
22. Schwarz, J. R., J. D. Walker, and R. R. Colwell. 1975. Deep-sea bacteria: growth and utilization of n-hexadecane at in situ temperature and pressure. *Can. J. of Microbiol.* 21:682-687.
23. Seki, H. 1976. Method for estimating the decomposition of hexadecane in the marine environment. *Appl. Environ. Microbiol.* 31:439-441.
24. Shaheen, D. G. 1975. Contributions of urban roadway usage to water pollution. U.S. Environmental Protection Agency, Report 600-2-75-004, Washington, D.C.
25. Smith, D. W., C. B. Fliermans, and T. D. Brock. 1972. Technique for measuring  $^{14}\text{CO}_2$  uptake by soil microorganisms in situ. *Appl. Microbiol.* 23:595-600.
26. Wakeham, S. G. 1977. Hydrocarbon budgets for Lake Washington. *Limnology and Oceanography*, 22:952-957.
27. Walker, J. D., H. F. Austin and R. R. Colwell. 1975. Utilization of mixed hydrocarbon substrates by petroleum-degrading microorganisms. *J. Gen. Appl. Microbiol.* 21:27-39.
28. Walker, J. D., J. J. Calomiris, T. L. Herbert and R. R. Colwell. 1976. Petroleum hydrocarbons: degradation and growth potential for Atlantic Ocean sediment bacteria. *Marine Biol.* 34:1-9.
29. Walker, J. D. and R. R. Colwell. 1974. Microbial degradation of model petroleum at low temperatures. *Microbiol. Ecology* 1:63-95.
30. Walker, J. D. and R. R. Colwell. 1976. Measuring the potential activity of hydrocarbon-degrading bacteria. *Appl. Environ. Microbiol.* 31:189-197.
31. Walker, J. D., R. R. Colwell, and L. Petrakis. 1975. Evaluation of petroleum-degrading potential of bacteria from water and sediment. *Appl. Environ. Microbiol.* 30:1036-1039.
32. Wang, C. H. and D. L. Willis. 1965. Radiotracer methodology in biological science, p. 294-298. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

33. Wanielista, M. P., R. N. Gennaro, J. H. Bell and J. W. Johnson. 1978. Shallow-water roadside ditches for stormwater purification, p. 3-10. State University System: Star program, Florida Technological University, Orlando, Florida.
34. Ward, D. M. and T. D. Brock. 1976. Environmental factors influencing the rate of hydrocarbon oxidation in temperate lakes. Appl. Environ. Microbiol. 31:764-772.
35. Zobell, C. E. 1973. Microbial degradation of oil: present status, problems and perspectives, p. 3-16. In D. G. Ahearn and S. P. Mereys (ed.), The Microbial degradation of oil pollutants. Louisiana State University Publ. no. LSU-SG-73-01, Baton Rouge.